Protective Effects of Seaweeds Against Liver Injury Caused By Carbon Tetrachloride And Trichloroethylene in Rats

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Abstract

This research is to investigate the potential protective effect of seaweed extracts against the carbon tetrachloride (CCl_4)- and trichloroethylene (TCE)-induced toxicity in male Sprague Dawley rats. Three species of seaweeds: *Myagropsis myagroides*, *Sargassum henslowianum* and *S. siliquastrum*, collected from Tung Ping Chau, Hong Kong were screened for their protective activity.

A single oral dosage of 1.25 ml/kg of 20% CCl_4 in corn oil was able to produce significantly elevated level of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activities together with the massive centrilobular necrosis and fatty accumulation in the histopathological examination. In the curative test of aqueous extracts of these fresh seaweeds at three different dosages (15, 30 and 60 mg/ml saline), *S. henslowianum* exhibited the most prominent hepatoprotective effect not only suppressed the elevation of SGPT and SGOT levels seen in CCl_4-toxicity in rats but also promoted the recovery or persevered the structural integrity of liver cells. However, *S. siliquastrum* exhibited significant protective effect only when the higher dosages (30 and 60 mg/ml saline) were applied. Based on the present preventive test, the protective function of all aqueous extracts of these seaweeds could not be demonstrated biochemically and histologically in the liver of the experimental animals.

The study of TCE toxicity test showed that the 1.25 ml/kg of 20% TCE in corn oil by intraperitoneal injection could cause significantly elevated level of SGPT and SGOT with no mortality of rat occurred. But the histopathological study could not

show markedly injury in the tissues of the liver and kidney. The pattern and organization of liver and kidney cells revealed no difference when compared with those of the control group administered with saline-vehicle only. The result of the one-time and two-time gavage oral dosage of TCE in the same dosage could not produce conspicuous increase of SGPT and SGOT. In the present test, it appeared that the main target site(s) of TCE might not be the liver or kidney. However, the actual target site(s), which could be used to explain the acute elevation of SGOT, was still unknown. Hence, 1.25 ml/kg of 20% TCE by i.p. (effective dose) was used as a toxin model to raise transaminase activity with the aim for the evaluation of protective effect of seaweed extracts against the TCE-induced acute elevated level of SGPT and SGOT only.

In the curative test of TCE effective dose, the extract *S. henslowianum* exhibited the most prominent protective effect against the elevation of SGPT and SGOT levels, whereas *S. siliquastrum* exhibited significant protective effect only at the higher dosages applied. In the preventive test, all seaweed extracts demonstrated significant effect in reducing the elevated level of SGOT only. Nevertheless, the effect was not as good as that of curative one.

In another experiment, dimethyl sulfoxide (DMSO) and N-acetylcysteine (NAC) could be demonstrated to have protective effect against both the CCl_4-and TCE-induced toxicity in the curative test. Especially in the NAC treatment at the higher dosages, it significantly reduced the SGPT and SGOT levels. This is in line with the convincing evidence from histopathological examinations in CCl_4-induced hepatotoxicity rats. Moreover, the result obtained from biochemical and

histopathological tests were quite similar to that of *S. henslowianum* extract treatment against CCl_4-induced hepatotoxicity at the corresponding dosages. Nevertheless, the overall performance of DMSO and NAC in the preventive test showed a poor protective effect against both toxins-induced toxicity. Even at the highest dosage applied, the effect was not prominent.

In the primary test on the methanol extract of fresh seaweeds, oral administration of 30 mg/ml saline dosage of seaweeds' methanol extracts from *S. henslowianum* and *S. siliquastrum* could significantly reduce the CCl_4-induced acute elevation of the levels of SGPT and SGOT in rats. Histopathological study of liver tissue also showed signs of partial recovery or inhibition of the massive centrilobular necrosis and fatty accumulation induced by CCl_4. However, *M. myagroides* extract produced poor curative effect in lowering the levels of SGPT and SGOT. However, in the TCE-induced toxicity test in which the same treatment was performed, *M. myagroides* exhibited prominent protective effect in lowering both serum enzymes GPT and GOT. The result of biochemical analysis showed that the methanol extracts exhibited better effect than that of the aqueous extracts at the same dosage or even higher. However, there was no significant evidence of tissue recovery in the histological study. This showed that the action mechanism(s) of methanol extract of seaweeds *in vivo* were not the same as the aqueous one. Moreover, the possible active agent(s) was not the same either.

The present results indicate that the seaweeds under investigation may possess active component(s) in the aqueous and methanol extracts, which may act as an antidote to protect the toxicity induced by CCl_4 and TCE in rats. The active

component(s) which may contain antioxidant or free radical scavenging activity to inhibit or prevent the toxicity produced. The actual protective mechanism is still speculative at this stage. Further investigation is necessary to clarify and characterize the possible active component(s) in the extracts. Hopefully, the active component(s) may be developed as useful antidotes to treat injury caused by CCl_4- and TCE-induced toxicity.

摘要

這項研究是以海藻抽取物作研究的對象，測試其潛在的保護作用，研究其對於 CCl_4 及 TCE 在大老鼠體內所產生的毒性之抑制作用。於這個測試中，用作研究的海藻有三種，它們分別是從香港東平洲採摘得來的褐藻類：(*Myagropsis myagroides, Sargassum henslowianum and S. siliquastrum*)。

以 1.25 ml/kg 劑量的 20% CCl_4（強飼）能使血液中的血清穀氨丙酮轉氨酶(SGPT) 及血清穀氨丁酮乙轉氨酶(SGOT) 有明顯的急性提升。同時，在細胞組織病理學的研究中亦發現此劑量的 CCl_4 亦能使肝臟組織產生大量的中央小葉細胞壞疽和累積過多的脂肪。於補救測試 (curative test) 顯示，用了三個不同劑量(15，30 和 60 mg/ml 鹽水) 的海藻水性抽取物進行試，其中褐藻 (*S. henslowianum*) 表現出最顯著的保肝作用，它不但能抑制 CCl_4 所引致大老鼠血液中的 SGPT 和 SGOT 的提升，並且能促進肝細胞的復元或穩固肝細胞組織結構上的完整。然而，褐藻 (*S. siliquastrum*) 卻要在較高的劑量 (30 和 60 mg/ml 鹽水) 才能展示出明顯的保護作用。不過，即使轉氨酶有所降低，肝臟細胞組織仍然是有非常明顯的損傷。此外，在預防測試（preventive test）中，所有海藻水性抽取物都不能於生化及肝臟組織研究中證明有保護肝臟的效用。

於毒性劑量測試中，證實腹腔注射 1.25 ml/kg 的 20% TCE 能引致 SGPT 和 SGOT 的顯著提升，並且沒有導致大老鼠死亡。但是，從細胞組織病理學的研究中，老鼠的肝臟及腎臟組織並沒有明顯的損傷變化；組織的結構及排列跟控制組別的大老鼠（ 只是供給鹽水及媒介物 ） 沒有任何分別。此外，於同等劑量 TCE （一劑及兩劑）的測試中(強飼)，亦不能引致 SGPT 和 SGOT 的明顯提升。於這項 TCE 毒性測試中，可以推斷 TCE 所損害的主要器官並不是肝臟及腎臟。但是， 哪一個器官因 TCE 毒性而引起 SGOT 的提升仍然是不清楚。因此，腹腔注射 1.25 ml/kg 的 20% TCE

(有效劑量) 只可以作為一個毒性的模型，以用來測試海藻抽取物對抑制 TCE 所產生 SGPT 和 SGOT 之急性提升的影響。

在補救測試中，海藻 (*S. henslowianum*) 對抑制 TCE (有效劑量) 所引起的 SGPT 和 SGOT 的急性提升有顯著的保護作用；然而，海藻 (*S. siliquastrum*) 只能於較高的劑量才能產生明顯的保護作用。但是，在預防測試中，所有海藻抽取物只有對於 SGOT 才有明顯的抑制作用。不過，效果並不及補救測試的好。

於其他的預防測試中，實驗証明二甲基氧化硫 (DMSO) 和 N-乙酰半胱氨酸 (NAC) 對 CCl_4 和 TCE 所產生的毒性有抑制的作用，特別是於高劑量的 NAC 測試中，它對 SGPT 和 SGOT 的抑制尤其顯著。這些抑制的效果大可與細胞組織病理學研究中，NAC 對 CCl_4 所引致大老鼠肝臟毒性的抑制結果互相吻合。這些結果跟海藻 (*S. henslowianum*) 抽取物的保肝作用有所相似。然而，DMSO 和 NAC 在預防測試中均對抑制上述兩種毒素表現出不理想的保護效果；即使給予最高的劑量，效果也不顯著。

於初步測試用甲醇提取的海藻研究中，發現用劑量 : 30 mg/ml 25% DMSO 的海藻（*S. henslowianum* 和 *S. siliquastrum*） 能對 CCl_4 所引致 SGPT 和 SGOT 的急性提升有著明顯的抑制作用， 此外，它亦能對肝臟細胞組織的中央小葉壞疽和脂肪累積有著緩和的作用。但是，海藻(*M. myagroides*) 抽取物在這項補救測試中，對於降低 SGPT 和 SGOT 卻表現出頗差的效果。不過，在 TCE 所產生的毒性測試中，相同的劑量卻表現出最顯著的保護效果。從生化測試的分析結果中可以大概知道，用甲醇提取的海藻比用水提取 (同樣或甚至更高的劑量) 的效果更好。不過，在細胞組織分析中卻沒有非常明顯的復元跡象。因此，用甲醇和水分別所提取的海藻抽取物可能有所不同，故此於大老鼠體內中亦起著不同的作用。

從研究結果顯示，所研究的海藻可能具有某類有用的天然物質，以抑制 CCl_4 和 TCE 所產生的毒性。這類物質可能擁有抗氧化或清除自由基的作用；以抑制或防止毒性的產生。於現階段，只可用純理論性推測來解釋這項保護機制。日後有待更深入的研究去加以闡明，並找出抽取物的有效成份，而這些成份可能有機會發展為抑制 CCl_4 和 TCE 所產生的毒性和損傷的解毒劑。

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Chapter 1 INTRODUCTION

It is a well-known fact that the Chinese people have been using seaweeds for various medicinal purposes such as for treatment of goiter and scrofula. Early records of medicinal seaweeds appeared in Chinese literature about two thousand years ago (Tseng & Chang, 1984). Some brown macroalgae were reported to have certain chemical compounds such as organic acids and phenolic compounds, especially polyphenols or tannins which were shown to have antimicrobial activity (Glombitza, 1979; Rosell & Srivastava, 1987). Several other studies have reported that marine algae (microalgae or macroalgae) contain various biologically active compounds (Baker, 1984; Tutour, 1990; Lee et al., 1996; Harada et al., 1997). Furthermore, some macroalgae, especially brown seaweeds, have been shown to have antioxidant activities in their extracts (Tutour, 1990; Lee et al., 1996). However, scientific studies on the protective effect of Hong Kong seaweeds in acute liver damage are lacking and in this investigation the local seaweeds (brown macroalgae): *Myagropsis myagroides* **(Fig. 1.1)**, *Sargassum henslowianum* **(Fig. 1.2)** and *S. siliquastrum* **(Fig. 1.3)** collected from Tung Ping Chau, Hong Kong were selected mainly to study their protective effects on carbon tetrachloride (CCl_4)- induced acute hepatotoxicity in male Sprague Dawley rats. In addition, acute hepatotoxic effect of seaweeds were also investigated to study any adverse or acute toxic effect on liver. In this study, aqueous extracts of seaweeds were mainly used for the test. Besides, methanol extracts of seaweeds were also used for preliminary investigation only.

Fig. 1.1 Fresh seaweed sample (young *Myagropsis myagroides*: S#3)

Fig. 1.2 Fresh seaweed sample (young *Sargassum henslowianum*: S#2)

Fig. 1.3 Fresh seaweed sample (young *Sargassum siliquastrum*: S#4)

In order to investigate the hepatoprotective effect of seaweeds' extract. Acute liver injuries were induced by chemicals, such as CCl_4, which has been used as a model hepatotoxin for many years to study the presence of hepatoprotective agents in experimental animals (Montilla et al., 1990; Gilani & Janbaz, 1995a, b and c; Jazbaz & Gilani, 1995; Gilani et al., 1996; Lin et al., 1996; Hase et al., 1997; Jeong et al, 1997; Wong et al., 1999). CCl_4 is a well-known hepatotoxin and an environmental contaminant which causes centrilobular necrosis and fatty accumulation in the liver accompanied by the elevated level of transaminases activity (SGPT and SGOT) in blood (Recknagel, 1967; Klaassen & Plaa, 1969; Harris et al., 1982). Elevated serum level of enzymes that are uniquely concentrated in the liver, especially SGPT, are reliable reflections of hepatic injury (Zimmerman & Seeff, 1970). Traditionally, the combined microscopic and biochemical techniques are important to establish the toxic or nontoxic character of a test compound at any early stage of investigation (Iglesia et al., 1982). As a result, if either the gross pathological changes or the severity of histopathology were graded and compared to the elevation in transaminases, there was a very good correlation between the elevation in transaminase activity and the severity of the lesion (Balazs et al., 1961; Plaa et al., 1982).

Owing to the widespread usage of tichloroethylene (TCE), it has been found as a contaminant in ground and surface water supplies (Murray & Riley, 1973). Even in extremely isolated areas, its toxicity presents a serious problem in both occupational and environmental health (Larson & Bull, 1989). However, the hepatotoxicity of TCE is not well established and the data are relatively inconclusive (USEPA, 1985; Waters et al., 1977). Nevertheless, conspicuous hepatotoxic response of TCE can be evaluated by the determination of serum transaminase activities 24 h after an

intraperitoneal injection pretreatment with cytochrome P-450 inducer, phenobarbital (Rouisse & Chakrabarti, 1986). In the present study, toxicity of TCE was investigated via oral and intraperitoneal routes of administration to find the possible elevated levels of serum transaminases without the pretreatment of phenobarbital. Furthermore, whether the main target site of TCE is the liver or not was also investigated. The result showed the effective dose of TCE which could induce the acute elevated level of SGPT and SGOT. Hopefully, the induced elevated levels of serum transaminases may be used as a toxin model for the hepatoprotective study of seaweed extracts similar to that of the CCl_4 model. In addition, the additional one seaweed (red species), *Galaxaura* sp. **(Fig. 1.4)**, was also used for the hepatotprotective test in TCE model.

In addition, N-acetylcysteine (NAC) and dimethyl sulfoxide (DMSO) are both reported to have antioxidant properties to relieve the CCl_4-induced toxicity in mice (Achudume, 1991; Kröger et al., 1997). In this research, they were selected for the study of their possible hepatoprotective effects on CCl_4 and TCE-induced toxicity in rats. Generally, it is likely that potent antiperoxidative agents can protect the rats' liver by preventing a CCl_3-induced peroxidative degradation of membranes (Yasuda et al., 1986). In fact, many hepatoprotective agents have a remarkable antiperoxidative action, which is considered to be at least one of the action mechanisms for their hepatoprotective effect (Castro et al., 1973; Yasuda et al., 1980). Yet, scientific reports on their effect against liver damage in rats are lacking. Moreover, antidotal effect of DMSO against CCl_4 is still inconclusive (Siegers, 1978; Achudumme, 1991).

Fig. 1.4 Fresh seaweed sample (*Galaxaura* sp.: Gal)

Thus, the present project aims to further conclude the hepatoprotective effect of DMSO and NAC against CCl_4-induced hepatotoxicity in rats. In addition, their potential protective action against the effective dose of TCE-induced toxicity was also investigated.

The objectives of this research are summarized as follows:

1. to adopt a suitable dosage of CCl_4 as a toxin model for screening hepatoprotective substances.
2. to study the protective effect of aqueous seaweed extracts against CCl_4-induced hepatotoxicity.
3. to test the possible acute hepatotoxic effect of aqueous seaweed extracts.
4. to investigate the toxicity of TCE in oral and i.p. routes in rats.
5. to elucidate whether the liver is the main target site of TCE toxicity.
6. to identify the effective toxic dose of TCE as a toxin model for hepatoprotective substance screening.
7. to study the protective effect of aqueous seaweed extracts against TCE-induced toxicity.
8. to study the potential protective effect of DMSO and NAC against CCl_4- and TCE-induced poisoning in rats.
9. to test the protective effect of methanol seaweed extracts against CCl_4- and TCE-induced poisoning in rats by curative mode.

Chapter 2 LITERATURE REVIEW

2.1 Toxicology.

Toxicology is defined as the study of the adverse effects of substances on living organisms. The adverse effects include their biochemical, cellular and molecular mechanisms of action (Klaassen and Eaton, 1991). The mode of action of toxic substances in the interaction with cellular components, and at the molecular level with structural proteins and other macromolecules, enzymes and receptors, and the types of toxic response produced, are key factors to determine the toxicity effects (Timbrell, 1994). Actually, different kinds of toxicants possess different modes of action, target organs and potencies. In order to study the hepatoprotective effect of seaweed, N-acetylcysteine and dimethyl sulfoxide, carbon tetrachloride was used as a toxic model to induce acute toxicity (short term) to the liver. The liver is the critical organ for metabolism, detoxification, and elimination of many chemicals that may be absorbed. As such, it is a likely target organ for toxic effects (Plaa, 1991). In addition, acute toxicological studies of trichloroethylene were also conducted in the present study. Hopefully, trichloroethylene may also be used as a toxic model as same as carbon tetrachloride for the screening of hepatoprotective substance.

2.1.1 Acute toxicity test.

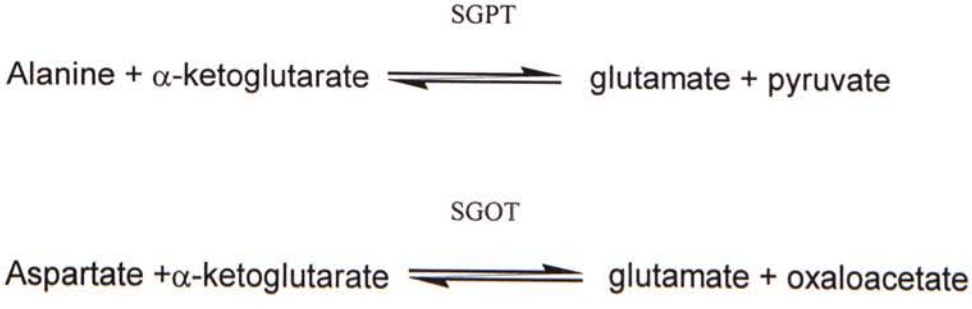
The definition of acute toxicity is the adverse effects occurring within a short time of administration of a single or multiple dosages of a substance given within 24 hours (Organization for Economic Cooperation and Development, 1981). Acute toxicity of carbon tetrachloride to the liver has been extensively studied during the

past 40 years (Rechnagel, 1967; Recknagel et al., 1989). The suitable dosage (found to be 1.25 ml/kg body weight) of carbon tetrachloride is applied to the rat to generate acute toxicity in a severe centrilobular type of necrosis and fatty degeneration of the liver with the acute elevated levels of SGPT and SGOT (Slater, 1966). In the present study, this dosage was used for the study of hepatoprotective effects of seaweed extracts and other substances. On the other hand, the hepatotoxicity of trichloroethylene is not well established and the data are relatively inconclusive (Waters et al, 1977; USEPA, 1985; Borzelleca et al, 1990;). Therefore, the acute hepatotoxicity test of trichloroethylene were studied in different aspects.

2.1.2 Biochemical analysis.

Animal serum contains many different kinds of transaminases. Biochemical analysis of blood serum is vital to evaluate target organ toxicity and provides information to determine the injuries on the target organ. Measurement of enzyme levels of the serum permits detection of hepatic toxicity with far less labor than that required for other biochemical tests or histologic analysis. The value of determination of serum transaminases as an aid in the diagnosis of necrosis of hepatic cells has been well established (Reitman & Frankel, 1957; Amador & Wacker, 1962; Amador et al., 1967). Serum glutamate pyruvate transaminase (SGPT) was also referred to as alanine aminotransferase (ALT) and serum glutamate oxaloacetate transaminase (SGOT) was also referred to as aspartate aminotransferase (AST). SGPT catalyzes the transfer of an amino group from the amino acid alanine to oxoglutarate (keto acid) to produce glutamate and SGOT catalyzes the transfer of an amino group from the amino acid aspartate to oxoglutarate (keto acid) to form glutamate. SGPT and SGOT catalyze the reactions as follows (Reitman & Frankel,

1957):



Normally, SGPT and SGOT can be found in cellular cytosol. They are present in blood in relatively low concentrations (Zimmerman, 1982). SGOT is a cytoplasmic and mitochondrial isoenzyme and is widely distributed in tissues and cells including hepatocytes, kidney cells, red blood cells, myocardium, pancreas and skeletal tissue (Nakamura et al., 1965; Amador et al., 1966; Zimmerman & Seeff, 1970). A raised activity of SGOT may be due to liver disease, myocardial disease, skeletal muscle disease, renal infarction, haemolysis or hypothyroidism (Jones & Berk, 1979). SGPT exists mainly in the cytosol and is specific for hepatocytes (Ooi, 1996). As a result, an elevated level of SGPT would be a good indicator of hepatocellular injury (Balazs et al., 1962; Jones & Berk, 1979). In the rats, SGPT is almost as sensitive as SGOT (Zimmerman & Seeff, 1970). This biochemical analysis method was well adopted in the recent pharmacological studies of hepatoprotective or antidotal effects of potential substances (Montilla et al., 1990; Gilani & Janaz, 1995a; Gilani & Janaz, 1995b; Gilani & Janaz, 1995c; Janbaz & Gilani, 1995; Gilani et al., 1996; Lin et al., 1996; Ooi, 1996; Jeong et al., 1997; Wong et al., 1999).

2.1.3 Organ weights.

In the present study, organ weights of the liver and kidney from the test animals

were measured after 24 hours experimental time and compared with those of control animals. This provides a primary screening of injured organs which may be subjected to further histopathological study.

2.2 Histology.

2.2.1 Light microscopy.

Light microscopy (LM) is a basic and traditional method for studying and defining the type of toxic hepatic injury (Rouiller, 1964; Zimmerman, 1978). It gives the yardstick against which other abnormalities can be measured (Cutler, 1974). The significance of adverse biochemical changes as indices of hepatic injury should be judged by the supporting evidence of remarkable necrosis or steatosis (Curtis, 1972). As a result, it demonstrates a frank lesion (Zimmerman, 1982). However, LM cannot quantify the degree of liver damage. These qualitative observations do not take into consideration any parameter regarding the fibrosis because they do not provide numerical data (Gaudio et al., 1993). It provides only a crude estimation.

2.2.2 Electron microscopy.

Electron microscope is an important instrument to study the ultrastructural abnormalities in the experimental hepatotoxicity (Rouiller, 1964; Schaffner and Popper, 1975). It provides a much earlier demonstration of hepatocyte injury and permits the recognition of injury too subtle to be achieved by light microscopy (Zimmerman, 1982). It can also yield clues to the mechanisms of hepatotoxicity by studying the structural changes and rate of its development induced by toxic agents (Zimmerman, 1982). Two types of electron microscope were used in this study·

Jeol-5300 SEM scanning electronic microscope (SEM) and Jeol JEM-1200 EX transmission electron microscope (TEM).

In this study, light microscopy study was performed on the liver and kidney of the treated rats. Hematoxylin and eosin staining were used to stain the cells. Besides, TEM and SEM were also used to study the liver cells of the carbon tetrachloride- and trichloroethylene-treated rats only (toxin control model).

2.3 Tissue injury.

2.3.1 Free-radical mechanisms.

Tissue injury can occur in many ways. An important way of injury is the production of free radical intermediates to trigger an expanding network of multifarious disturbances (Slater, 1984). Free radicals are defined as molecules or molecular fragments with one or more unpaired electron (Halliwell & Gutteridge, 1984; Slater, 1984). Free radicals can be positively charged, negatively charged or electrically neutral in nature. The unpaired electron causes certain unique properties to the free radical such as paramagnetism. In general, typical reactions of free radicals are (a) electron donation (from a reducing radical) and electron acceptance (for an oxidizing radical); (b) hydrogen abstraction; (c) addition reactions; (d) disproportionations; and (e) self-annihilation reactions as follows (Slater, 1984):

(a) $OH^- + RS^- \longrightarrow OH^- + RS\cdot$

(b) $CCl_3\cdot + RH \longrightarrow CHCl_3 + R\cdot$

(c) $CCl_3\cdot + CH_2{=}CH_2 \longrightarrow CH_2(CCl_3)\text{-}CH_2$

(d) $CCl_3 \cdot + CCl_3 \cdot \longrightarrow C_2Cl_6$

(e) $CH_3CH_2 \cdot + CH_3CH_2 \cdot \longrightarrow CH_2{=}CH_2 + CH_2\text{-}CH_3$

Free radicals generated by hepatic metabolism or by drug biotransformation reactions can also cause cell injury by oxidation of unsaturated fatty acids of phospholipids (Marzella & Trump, 1991). The chemical reactivity of free radicals is usually high to cause direct or indirect biological damaging effects. For example: Free radicals react with the double bonds of unsaturated fatty acids and with sulphydryl groups in proteins. Hydroxyl radical is primarily involved in lipid damage. It can react with the unsaturated bonds to produce a lipid free radical, which in turn to form a lipid peroxide by the reaction with oxygen (Recknagel, 1967; Recknagel et al., 1989). The lipid peroxide formed can act as a free radical to react with a further unsaturated bond of a fatty acid to establish an autocatalytic chain reaction (MacSween & Whaley, 1992). As a result, the membrane permeability change is due to the damage of phospholipids (Slater, 1966). In addition, free radicals react with sulphydryl groups to form disulphide bonds with cross-linking of proteins. This may affect the enzyme function or produce focal increases in membrane permeability (Slater, 1984).

2.3.2 Lipid peroxidation.

Lipid peroxidation is defined as the metabolism of lipids through pathways involving formation of intermediates such as lipid peroxides, hydroperoxides and endoperoxides (Recknagel et al., 1989). The peroxidation of polyunsaturated fatty acids or membrane phospholipids are a feature of many types of cell injury in which free radical intermediates are produced in excess of local defence mechanisms to obtain sufficient reactivity to abstract a hydrogen atom (Halliwell & Gutteridge,

1984; Slater, 1984; Groot et al., 1988). The equations (1) below shows that hydrogen atom abstracted from the lipid leaves behind an unpaired electron. The carbon radical in that polyunsaturated fatty acid tends to be stabilized by a molecular rearrangement. The conjugated diene obtained which reacts with oxygen to give a hydroperoxy radical in equation (2). Hydroperoxy radicals formed abstract hydrogen atoms from other lipid molecules to continue the self-propagating chain reaction of lipid peroxidation. The hydroperoxy radical combines with the hydrogen atom that it abstracts to form a lipid hydroperoxide in equation (3). This process is commonly referred to as "propagation of lipid peroxidation" (Recknagel & Glende, 1977). For example, the bioactivation of carbon tetrachloride and the initiation of the self-propagating lipid peroxidation, working in tandem, destroy the cellular membranes leading to cell death (Mehendale, 1991).

Equation (1): $\text{Lipid-H} + {}^{\cdot}\text{OH} \longrightarrow \text{Lipid}^{\cdot} + H_2O$

Equation (2): $\text{Lipid}^{\cdot} + {}^{\cdot}O_2 \longrightarrow \text{Lipid-}O_2{}^{\cdot}$

After molecular rearrangement

Equation (3): $\text{Lipid-}O_2{}^{\cdot} + \text{Lipid-H} \longrightarrow \text{Lipid-}O_2H + \text{Lipid}^{\cdot}$

2.4 Carbon tetrachloride (CCl_4).

Carbon tetrachloride (CCl_4) is a simple molecule **(Fig. 2.1)** and a well-known hepatotoxin, which has been extensively studied (Slater, 1966; Recknagel, 1989). The primary use of CCl_4 is in the chemical manufacture of dry cleaning agent, fluorocarbon refrigerants, organic solvents, and aerosol propellants (Trevethick, 1980). Accumulating evidence over the years has shown CCl_4 to be one of the most toxic common solvents (Hamilton & Hardy, 1974). By the 1930s, it was widely used as a dry cleaning agent and constituent of fire extinguishers (Nielsen & Larsen, 1965;

USEPA, 1984). The banning of use of CCl_4 in fire extinguishers by the early 1960s was due to the recognition of its fatalities resulting from thermal decomposition. Moreover, industrial use of CCl_4 began declining in the 1950s as its acute toxicity and fatalities became well known. Therefore, it has been banned in all products except where it is an unavoidable by product. As a result, exposure is limited to its industrial use in the production of solvents, aerosol propellants and refrigerants (USEPA, 1984). However, it has lost its early predominance as a cheap nonflammable chlorinated solvent for use in degreasing and dry cleaning. Less toxic agent such as trichloroethylene has served as admirable substitutes (Hamilton & Hardy, 1974).

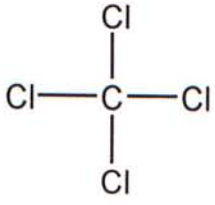


Fig. 2.1 Structural formula of carbon tetrachloride (CCl_4)

2.4.1 Mechanisms of carbon tetrachloride toxicity.

The mechanism of CCl_4 hepatotoxicity has been studied in great detail by Rechnagel (1967). The collected evidence indicates that the two prime pathologic observations, fatty degeneration and centrilobular necrosis, have distinct pathogenesis. Although a clear understanding of its mechanisms in hepatotoxicity has not yet been reached, several reviews describing the mechanism of CCl_4 toxicity have been published (Slater, 1966; Recknagel, 1967; Recknagel et al., 1989; Mehendale, 1991). Early investigations proved that the leading theory for the CCl_4 hepatotoxicity is initially catalyzed by cytochrome P-450 in the endoplasmic reticulum (ER) of hepatocytes (Recknagel, 1967; Slater, 1984).

It has been well known for many years that CCl_4 is metabolized by the monooxygenase system of the ER. The enzyme system acts as a reductase to catalyze one-electron reductive dehalogenation, cleavage of C-Cl bond to yield trichloromethyl and monoatomic chlorine free radicals intemediates (Recknagel et al., 1989; Wang et al., 1996) **(Fig. 2.2)**. The trichloromethyl radical ($\cdot CCl_3$) may then react with oxygen to give the trichloromethylperoxy radical ($\cdot CCl_3O_2$). Alternatively, the $\cdot CCl_3$ can abstract a hydrogen atom from polyunsaturated lipids and thereby form a lipid radical and a stable product, chloroform ($CHCl_3$). The lipid radical can then proceed to react with other cellular constituents by oxygen addition and cause a cascade of disturbance with the cell, including peroxidation of lipids in endoplasmic reticulum (Packer et al., 1978; Recknagel et al., 1989). Alternatively, covalent binding of the $\cdot CCl_3$ to liver microsomal proteins and lipids may presumably occurr (Slater, 1966; Packer et al., 1978; Slater, 1984). The $\cdot CCl_3O_2$ is believed to be the reactive metabolite responsible for lipid peroxidation (Recknagel et al., 1989). However, various studies have suggested that although the initiating event may be the formation of the trichloromethyl radical, this is not the major cause of damage (Packer et al., 1978; Recknagel et al., 1989; Slater, 1984). As the destructive range of $\cdot CCl_3$ and $\cdot CCl_3O_2$ to the hepatocyte is restricted due to their limited spreading ability from the original sites (Recknagel et al., 1989), the extensive hepatotoxicity of CCl_4 is probably augmented by the formation of other products in overall process (Slater, 1984). Moreover, it has become clear that the presumably existence of a cascade of secondary mechanisms is evoked by the initial events of CCl_4 metabolism, and that the secondary mechanisms are responsible for ultimate plasma membrane disruption, degenerative effects on other substructural organelles of the cells and death of the cell (Recknagel et al., 1989; Shah et al., 1979).

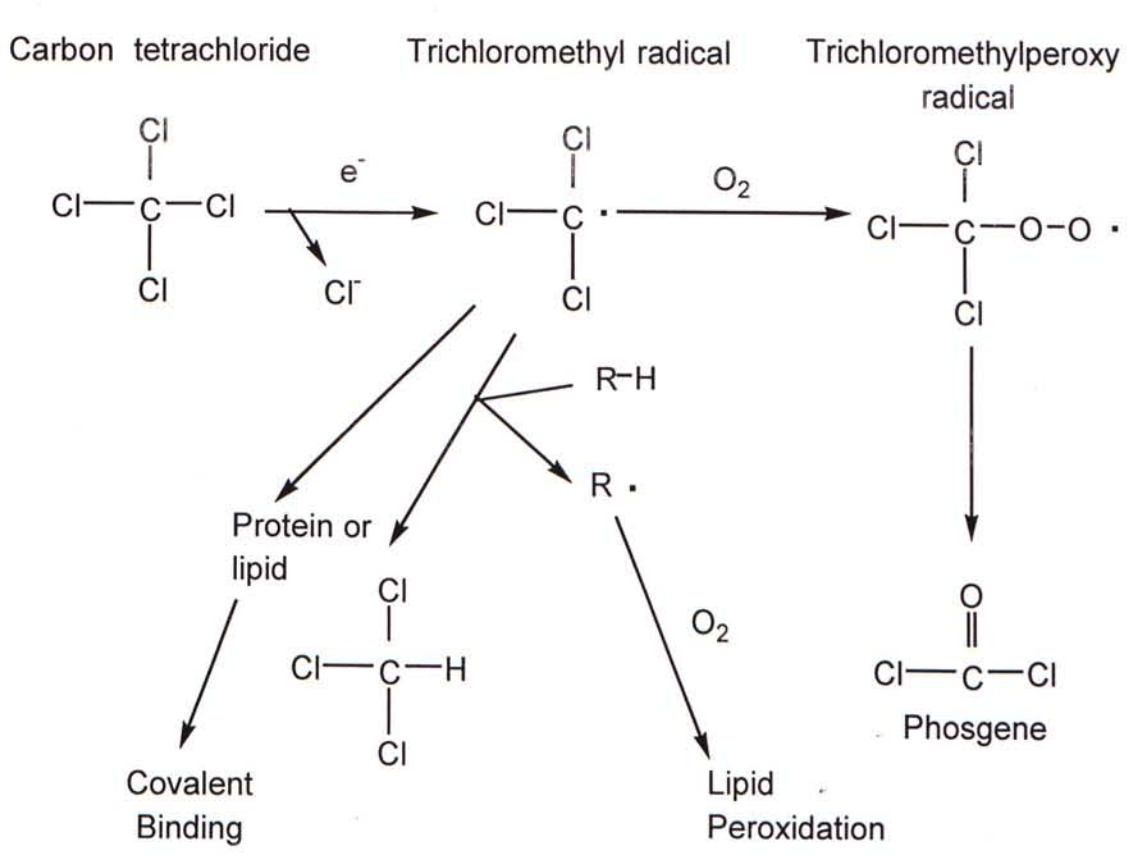


Fig. 2.2 The mechanism of microsomal enzyme mediated metabolic activation of carbon tetrachloride. (Adopted from Timbrell, 1994)

As indicated above in section 2.3.2, lipid peroxidation has been shown to play an important role in the hepatotoxicity of CCl_4. However, the role of free radicals or lipid peroxidation in CCl_4-induced nephrotoxicity remains unclear (Elfarra, 1993). Many common strains of laboratory rats are relatively resistant to the acute nephrotoxic effects of CCl_4 (Kluwe, 1981). In rats, $^{14}CCl_4$ administration results in covalent binding of the radiolabel to kidney and liver proteins and lipids (Villarruel et al., 1977). However, change in kidney morphology, for example, was not detected in rats after single or multiple dosages of CCl_4, though severe necrosis and cirrhosis were detected in the liver (Perassi & Martin, 1973; Soni & Mehendale, 1993). Moreover, no change in kidney morphology was visible at the level of light microscopy in Sprague-Dawley rats treated orally with 2.5 ml/kg CCl_4 though an increase in kidney weight, elevation of serum creatinine and a transient diuresis were evident (Striker et al., 1968).

2.5 Trichlororethylene (TCE).

Trichlororethylene (TCE) was first synthesized in 1864 by Fischer, patented in 1906, and introduced as a nonflammable narcotic in 1911 for medical practice (Defalque, 1961). **Table 1** shows chronologically the primary use patterns of TCE from discovery in the late 19th century through the proposed ban for use in food.

Trichloroethylene is an unsaturated halogenated hydrocarbon **(Fig. 2.3)** with an empirical formula C_2HCl_3 and a molecular weight of 131.4. It exists as a colorless, volatile liquid with a somewhat sweet odor and a sweet, burning taste resemble that of chloroform (Defalque, 1961; Elcombe et al., 1985). The solvent properties of TCE were not known until the early 1900s (Defalque, 1961). Because of its excellent fat solvent properties, chemical stability and relatively low acute toxicity (World Health Organization, 1985; Dekant et al., 1990), TCE has enjoyed widespread industrial use as industrial solvent for vapor degreasing of fabricated metal parts prior to painting, anodizing and electroplating (Elcombe et al., 1985; Kimbrough et al., 1985). Furthermore, it can be used as an household degreasing and cleaning agent (Allemand et al., 1978; Soni et al., 1998). It is also an extraction medium (for spices, coffee) and occasionally used in anesthesia as well as an ingredient in adhesives, typewriter correction fluid, paint removers and spot removers (Waters et al., 1977; Allemand et al., 1978; Larson & Bull, 1989; Hanioka et al., 1997). Historically, TCE has had a multitude uses: mordant and dye fixative, desizer for textiles, cauiking substance for shipbuilding, antifoaming agent, solvent for rubber and insecticides as well as defatting agent for skins and hides (Allemand et al., 1978). In addition, the reason why it is so common is due to its production price. Production of TCE is

inexpensive, and can be prepared by treating ethylene with chlorine to form tetrachlorethane , which reacts with lime slurry to give trichloroethylene (Defalque, 1961):

$$C_2H_2 + 2\ Cl_2 \longrightarrow C_2H_2Cl_4$$

$$2\ C_2H_2Cl_4 + Ca(OH)_2 \longrightarrow 2\ C_2HCl_2 + CaCl_2 + 2\ H_2O$$

However, TCE can easily decompose. In the presence of light, especially ultraviolet light (UV) and moisture, it decomposes to form acidic products including hydrogen chloride. Nevertheless, the decomposition is retarded by storage in amber colored bottles and addition of 0.01% thymol blue (Defalque, 1961).

Table 1. History of trichloroethylene (Adopted from Waters et al., 1977)

Year	History of trichloroethylene
1864	First prepared by Fischer
1906	First patent held by Konsortium Für Elektrochemische Industrie, Nünberg
1911	Narcotic properties discovered by Lehman
1914-1918	Limited use as a degreaser and solvent
1915	Trigeminal analgesia reported by Plessner
1920s	More widespread use in metal degreasing
1930s	Use spreads to dry-cleaning industry
1933	Jackson successfully anesthetizes dogs
1940s	Use in Great Britain as inexpensive, nonexplosive anesthetic
1945	Use as anesthetic spreads to the United States, does not gain widespread popularity
1960s	"Carbona-cult" solvent-sniffing
1966	Use as a solvent curtailed in Los Angeles County, California, as a result of evidence implicating TCE in severe smog formation
1975	Preliminary report indicating carcinogenicity
1975	Invocation of Delaney clause sought to ban all uses in foods
1976	FDA drafting order to ban TCE
1976	Other Chlorinated solvents proposed as substitutes: methyl chloroform, perchloroethylene, methylene chloride

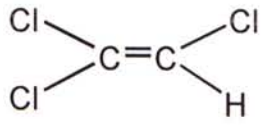


Fig. 2.3: Structural formula of trichloroethylene

Actually, as illustrated in the table below (**Table 2**), the general public is exposed to extremely low levels of trichloroethylene in water, air, and food.. But, trichloroethylene does not accumulate significantly in the food chain as it can be degraded by both abiotic and biotic processes. Its persistence in various environmental compartments is rather short, of the order of days or months rather than years (World Health Organization, 1985).

Table 2: Maximum observed concentration of trichloroethylene in environmental media (Adopted from World Health Organization, 1985)

Media	Maximum observed concentration
Open water reservoir	220 μg/litre
Industrial discharge water	200 μg/litre
Rain water	~ 1 μg/litre
Atmosphere	~ 40 $\mu g/m^3$
Dairy foods	10 μg/kg
Meat	22 μg/kg
Fats and oil	19 μg/kg

Owing to the medical and industrial use of trichloroethylene (TCE), the National Institute for Occupational Safety and Health (NIOSH) carried out a survey

in 1975, which indicated that nearly 3,000,000 industrial workers are exposed to TCE each year, often in massive dosages, and approximately 5,000 operating room personnel and dentists have regular contact (Waters et al., 1977). Occupational exposure of TCE is of concern because of the toxic effects of TCE observed in mice and rats (Waters et al., 1977).

2.5.1 Mechanisms of trichloroethylene toxicity.

Trichloroethylene is absorbed into the animal system by oral, dermal and inhalational routes (Hamilton & Hardy, 1974). According to some reports published, TCE passes across the gastrointestinal wall easily, as illustrated by many cases of poisoning following oral ingestion of TCE (Waters et al., 1977).

According to **Fig. 2.4**, oxidation of TCE through intermediates to chloral hydrate is mediated by a reduced nicotinamide adenine dinucleotide phosphate/oxygen ($NADPH/O_2$)-dependent reaction taking place in liver microsomes in a reaction requiring NADPH and oxygen (Byington & Leibman, 1965; Leibman, 1965). Moreover, chloral hydrate is known to be the common intermediate from which the major urinary metabolites trichloroacetic acid (TCA) and trichloroethanol (TCEtOH) are derived (Byington & Leibman, 1965). The reduction of chloral to TCEtOH is catalyzed by alcohol dehydrogenase and requires NADH. However, the oxidation of chloral hydrate to TCA requires NAD^+ and is catalyzed by chloral hydrate dehydrogenase (Sato et al., 1981).

The metabolism of trichloroethylene is thought to proceed via an unstable reactive intermediate mediated by hepatic microsomal mixed-function oxidase (MFO)

system which contains cytochrome P-450 (Reynolds and Moslen, 1977; Rouisse and Chakrabarti, 1986). TCE is metabolized by this cytochrome P-450 into a chemically reactive metabolite which reacts with, and binds to either glutathione or protein or undergoes spontaneous rearrangement to chloral hydrate leading to either tichloroethanol or to trichloroacetic acid. Based on the three reactions mentioned, the process of binding of TCE to protein may lead to the production of liver lesion (Allemand et al., 1978).

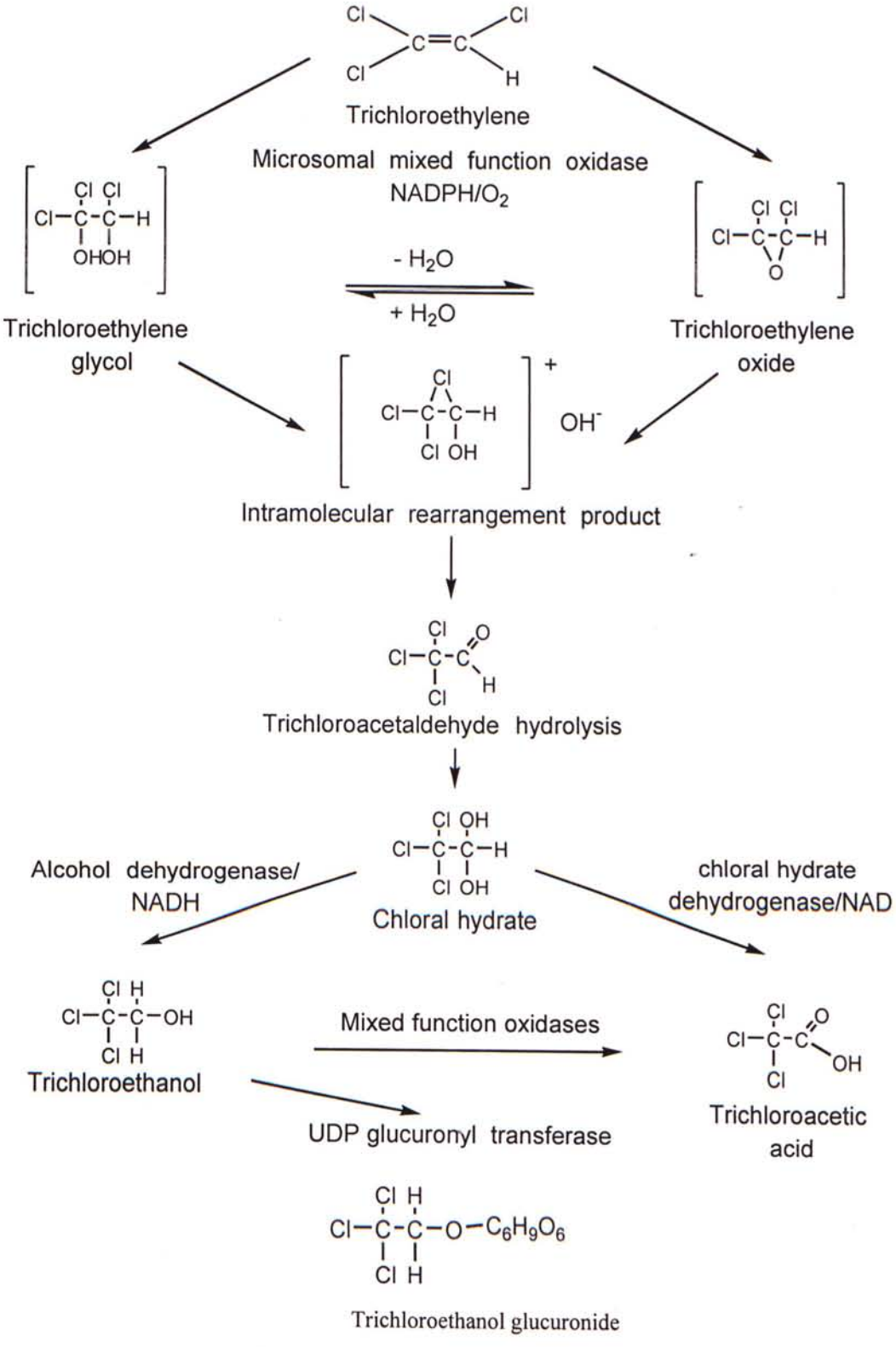


Fig. 2.4 Proposed intermediary metabolism of TCE

(Adopted from Waters et al., 1977)

Owing to the unstable properties, the chemically reactive metabolites are difficult to identify. Among those whose chemical structure is reasonably well established, there are a number of epoxides (Allemand et al., 1978). Besides, an epoxide intermediates (**Fig. 2.5**) is thought to occur. It is due to the initial oxidation product of TCE by the microsomal mixed-function oxidase system (Allemand et al., 1978; Byington & Leibman, 1965). The presumed trichloroethylene epoxide may directly undergo three different reactions as mentioned above. And, the reactive metabolite covalently binds to hepatic proteins may cause liver cell necrosis (Allemand et al., 1978).

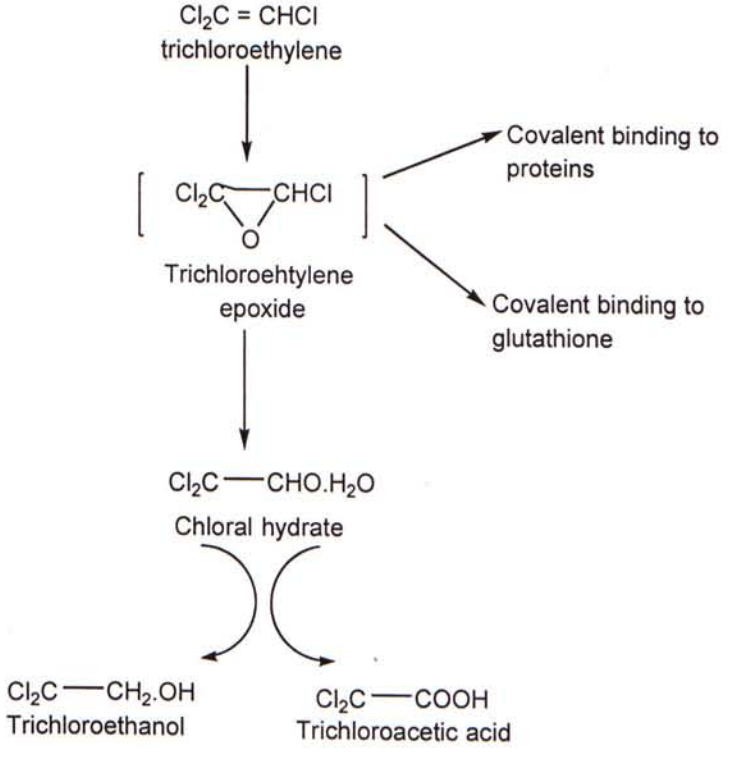


Fig. 2.5 Proposed metabolism of trichloroethylene. Trichloroethylene is metabolized by the microsomal mixed-function oxidase system to an unstable intermediate, probably trichloroethylene epoxide. The unstable intermediate may undergo three different reactions: 1) covalent binding to proteins, 2) covalent binding to glutathione or 3) spontaneous rearrangement to chloral hydrate which may be reduced to trichloroethanol or oxidized to trichloroacetic acid.

(Adopted from Allemand et al., 1978)

Although exposure to large amount of trichloroethylene can induce hepatic and renal injury, many studies in animals illustrate that TCE is only a weak hepatotoxin compared with carbon tetrachloride (CCl_4). Repeated exposure to excessive levels of TCE resulted in multiple episodes of chemical hepatitis followed by hypertension and cirrhosis (Thiele et al., 1982). The final episode of hepatitis was caused by trichloroethane. Based on recent animal and epidemiological studies, TCE has little if any hepatotoxic effect in the accepted range of occupational TCE exposure (Waters et al., 1977). Most of the studies show that conspicuous hepatotoxicity of TCE (dosage dissolved in corn oil administered intraperitoneally) develops only in rats pretreated with phenobarbital (Rouisse and Chakrabarti, 1986; Borzelleca et al., 1990). The hepatotoxicity of TCE is not well established due to the fact that the data are relatively inconclusive (Waters et al., 1977; USEPA, 1985).

2.6 Dimethyl sulfoxide (DMSO).

Dimethyl sulfoxide (DMSO) is a remarkable and controversial chemical compound. It is prepared by Alexander Saylzeff in 1866 (Leake, 1967). DMSO is dipolar aprotic solvent. So, it differs from water which is protic solvent, as its tendency to accept protons. It can be represented in the polarized form as follows **(Fig. 2.6)**:

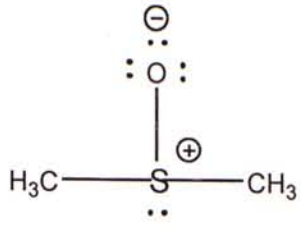


Fig. 2.6: Structure of DMSO in polarized form

Based on the structure, oxygen and sulfur have unshared electron pairs. As a result, DMSO has the ability to form either stable solvates by dipole-dipole interactions or solvent-solute associations by hydrophobic interactions (Rammler & Zaffaroni, 1967). Therefore, it is extremely hygroscopic and miscible with water. Furthermore, it is also employed to remove paint and varnish (Manahan, 1992). Owing to its broad solvent characteristics, DMSO is one of the solvents frequently used in pharmacological or toxicological experiments to improve the solubility of poorly soluble compounds (Siegers, 1978).

DMSO is an important chemical antioxidant (Achudume, 1991) with its anti-ischemic and anti-inflammatory properties (Lind and Gandolf, 1997). Besides, DMSO is reported to have cryoprotective and radioprotective properties (Sieger, 1978). It has been shown to protect the liver against injury produced by drugs and chemicals such as acetaminophen and carbon tetrachloride in mice only (Siegers, 1978; Achudume, 1991). But, DMSO is an inhibitor of cytochrome P-450-based biotransformation (Sieger, 1978; Jeffrey et al., 1988; Lind and Gandolf, 1997). So, the hepatoprotective effect illustrated above was most likely due to a lack of bioactivation of the compounds to their reactive toxic metabolites. In the present study, DMSO in different percentages were used to test its hepatoprotective effects in male Sprague-Dawley rats. Furthermore, the least hepatoprotective percentage was adopted as a vehicle to dissolve the poorly soluble methanol extracts of seaweeds for the preliminary test.

2.7 N-acetylcysteine (NAC).

N-acetylcysteine (NAC) was first introduced as a mucolytic agent in the 1960s and has since found wide use in clinical toxicology as a cytoprotective agent in acute acetaminophen (paracetamol) poisoning (Flanagan and Meredith, 1991). It is a well-known fact that acetaminophen has found increasing use in recent years as a substitute for aspirin, but it can cause liver damage in rats, mice and human (Kröger et al., 1997). Oral and intravenous administration of NAC can mitigate acetaminophen-induced hepatotoxicity by sulfate replenishment to restore intracellular GSH (Flanagan and Meredith, 1991). As GSH contributes significantly to the intracellular antioxidant defense system to be a powerful consumer of superoxide, singlet oxygen, and hydroxyl radicals (Miesel & Zuber, 1993). There is an evidence that NAC is an antioxidant to react with oxygen free radicals but is also used for the synthesis of cysteine and GSH (Sies, 1993). Moreover, it has been well reported that treatment with cysteine pro-drug such as NAC, cimetidine and meso-2,3-dimercaptosuccinic acid (DMSA) etc., can protect against acetaminophen hepatotoxicity in rats (Harman & Self, 1986; Speeg et al., 1985). Recently, NAC has been shown to have ability to inhibit inflammatory stimulations, including that of HIV replication (Roederer et al., 1993). Thus, it is suggested to be a potential drug for AIDS therapy and is used as an adjunct in the treatment of AIDS (Roederer et al., 1993). In the present study, hepatoprotective effects of NAC were studied in CCl_4 toxicity in rats. Furthermore, TCE toxicity was also investigated to test the protective effects produced from NAC.

Chapter 3 MATERIALS AND METHODS

3.1 Materials.

Hong Kong Seaweeds.

Four species of marine macroalgae: *Myagropsis myagroides, Sargassum henslowianum, S. siliquastrum* (brown macroalgae) and *Galaxaura* sp. (red macroalgae) were collected from Tung Ping Chau, Hong Kong by Prof. Put O. Ang, Jr. (Department of Biology, The Chinese University of Hong Kong) during spring-summer period (April-July/1997 and April-July/1998).

Animals.

Seven to eight weeks old male Sprague-Dawley rats, weighing 150g-250g, were obtained from the Laboratory Animals Service Centre, The Chinese University of Hong Kong. They were housed in a controlled-environment of 18-20 °C, humidity 54-56 % with 12-hour light-dark cycle and were supplied with standard rodent chows (Supastok Autoclavable Rodent Diet, Ridley Agriproducts, Australia) and tap water *ad libitum*. The food was withdrawn about 18 h before the experiment.

Chemicals.

Phosphate buffer, pH 7.5

Phosphate buffer was made by mixing 840 ml of 0.1 M disodium hydrogen orthophosphate (14.2 g Na_2HPO_4, anhydrous, Ajax, Australia) and 160 ml 0.1 M of

potassium dihydrogen orthophosphate (6.80 g KH_2PO_4, Ajax, Australia). The solution was adjusted to pH 7.5 by using 1M sodium hydroxide or 1M hydrochloric acid.

Sodium pyruvate

Calibration solution of SGPT and SGOT enzyme assay (1.5 mM of sodium pyruvate) was made by adding 0.017 g sodium pyruvate (Sigma, U.S.A) in 100 ml phosphate buffer (pH 7.5). The final solution was stored in the refrigerator at 2 ° to 6 °C and stable for about one month.

2,4-dinitrophenylhydrazine

The color reagent was prepared by adding 0.02 g of 2,4-dinitrophenylhydrazine (Sigma, U.S.A) to 100 ml of 1 N hydrochloric acid. The final solution was stored in the refrigerator at 2° to 6 °C and stable for about one month.

GPT substrate solution

The GPT substrate solution was prepared by mixing 4.45 g of dL-alanine (Sigma, U.S.A) and 0.066 g of α-ketoglutaric acid (Sigma, U.S.A) in 250 ml phosphate buffer (pH 7.5). The final solution was stored in refrigerator at 2° to 6 °C and stable for about one month.

GOT substrate solution

The GOT substrate solution was prepared by mixing 6.66 g of dL-aspartic acid, monosodium salt (Sigma, U.S.A) and 0.066 g of α-ketoglutaric acid (Sigma, U.S.A) in 250 ml phosphate buffer (pH 7.5). The final solution was stored in the refrigerator at 2° to 6 °C and stable for about one month.

Bouin's fluid

The Bouin's fluid was prepared by mixing 600 ml of picric acid, saturated (Hopkin and Willams, England), 160 ml of formalin, commercial (Ajax, Australia) and 40 ml of acetic acid, glacial (Ajax, Australia). The final solution was stored in room temperature ready for use.

3.2 Methods.

3.2.1 Acute hepatotoxicity test on aqueous seaweed extracts.

3.2.1.1 Preparation of aqueous extracts of seaweeds.

Aqueous extracts of fresh samples of *Myagropsis myagroides* (S#3), *Sargassum henslowianum* (S#2), *S. siliquastrum* (S#4) and *Galaxaura* sp. (Gal) were used for the investigation. For extraction, washed seaweeds were weighed and blended with distilled water. They were kept at 4 °C for 1 day and then filtered through cotton gauze. The filtrates were centrifuged at 23,700 g (12000 rpm) for 20 min. After centrifugation, the supernatants were freeze-dried. The resulting lyophilized powders were weighed and kept in the desiccator ready for use **(Fig 3.1 to Fig. 3.4)**.

3.2.1.2 Experimental protocol.

The experimental animals were divided into three groups, namely: the normal no treatment group (5 rats); the control group (5 rats) which received the vehicle (6.25 ml/kg, corn oil, Mazole, U.S.A.) orally by gavage oral administration **(Fig. 3.5)** and normal saline (10 ml/kg) was also administered orally 6 h after the vehicle treatment; the third group (5 rats) which was treated similarly to group 2 except that each seaweed extract was individually administered instead of saline for the primary evaluation of seaweeds' toxicity. Three dosages of seaweed extracts (150 mg/kg, 300 mg/kg and 600 mg/kg, dissolved in 10 ml saline) were used. Seven animals received one dosage of each seaweed extract except *Galaxaura* sp. extract (150 and 300

mg/kg). All treated animals were sacrificed 24 h after receiving the administration of the hepatotoxin (CCl_4).

3.2.1.3 Biochemical assays.

Enzyme activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) in blood serum served as indicators to illustrate the extent of hepatotoxicity in the rats. These enzymes, especially SGPT, are highly localized in hepatocyte cytosols.

The animals were anaesthetized with ether 24 h after the hepatotoxin treatment and blood (~5 ml) was withdrawn with sterile disposable syringes equipped with hypodermic needles from posterior vena cava **(Fig. 3.6)**. Serum was separated by centrifugation at 1,100 g (3000 rpm) for 15 min. Plasma was separated from the cells immediately to avoid interference caused by haemolysis as red blood cells also contain SGOT. The serum was then diluted 10 fold with 0.9% (v/v) saline. The serum enzyme levels of SGPT and SGOT were estimated according to the method of Reitman and Frankel immediately after the separation of plasma from the blood (Reitman and Frankel, 1957).

Fig 3.1 Aqueous seaweed extract (*Myagropsis myagroides*: S#3)

Fig 3.2 Aqueous seaweed extract (*Sargassum henslowianum*: S#2)

Fig 3.3 Aqueous seaweed extract (*Sargassum siliquastrum*: S#4)

Fig. 3.4 Aqueous seaweed extract (*Galaxaura* sp.: Gal)

Fig. 3.5 The photo of gavage oral administration by the intragastic tube

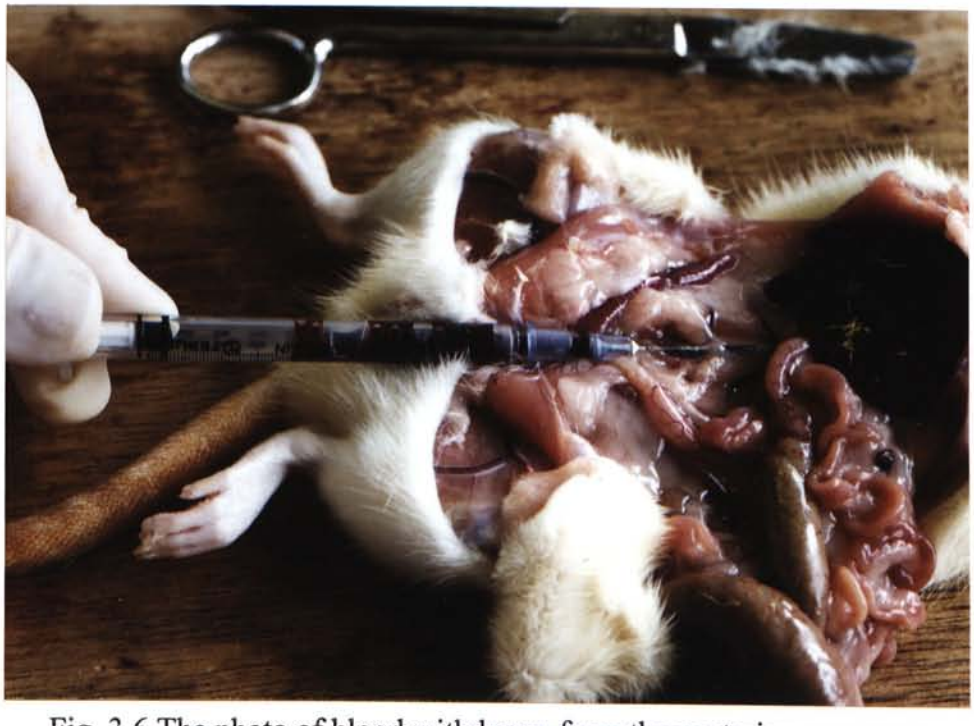


Fig. 3.6 The photo of blood withdrawn from the posterior vena cava

3.2.1.4 Organ weights.

The livers and kidneys of each treatment group were dissected out from the animals' bodies followed by the measurement of liver and kidney weight.

3.2.1.5 Histopathological examination.

(i) Light microscopy

A portion of the median lobe of the liver and left kidney were fixed in Bouin's fluid for 24 h and dehydrated though an ascending series of different percentage of ethanol; cleared in xylene. The liver and kidney specimens were then embedded in paraffin (melting point 56-58 °C), cut to 5-7 μm thickness by a microtome, and stained with Mayer's hematoxylin and eosin (H&E) for histopathological examination under light microscope. Detailed procedures for the histopathological processing are outlined in the Appendix B.

3.2.1.6 Statistical analysis.

For statistical analysis of the data, the Student's t-test and ANOVA were used to compare the levels of SGPT and SGOT in different treatments. All results are expressed in arithmetical means ± standard errors mean. The data were regarded as significance and very significance if $p< 0.05$ and $p< 0.005$ respectively. A two-way ANOVA was used to assess the difference in the effect of different treatments and their concentrations on affecting the levels of SGPT and SGOT. Where significant differences have been detected, a Tukey HSD Test was used to identify the group(s)

responsible for such differences.

3.2.2 Curative and preventive tests of seaweed aqueous extracts against the CCl_4-induced hepatotoxicity.

3.2.2.1 Preparation of aqueous extracts of seaweeds.

Aqueous extracts of fresh samples of *Myagropsis myagroides* (S#3), *Sargassum henslowianum* (S#2) and *S. siliquastrum* (S#4) were used for the investigation. For extraction, the method used was the same as that described in the section 3.2.1.1.

3.2.2.2 Experimental protocol (Fig. 3.7).

Carbon tetrachloride produces hepatotoxicity when taken in suitable dosage (1.25 ml/kg) (Slater, 1966). The 20% (v/v) CCl_4 (Ajax Chemicals, Australia) was made by dissolving CCl_4 in corn oil (Mazola, U.S.A.) and was administered orally to the stomach of the rats through an intragastric tube.

The experimental animals were divided into four groups, namely: the normal no treatment group (10 rats); the control group (10 rats) which received the vehicle, corn oil (Mazole, U.S.A.) by gavage oral administration in the dosage of 6.25 ml/kg and normal saline (10 ml/kg) was administered orally 6 h after the vehicle treatment; the toxin control group (10 rats) which received carbon tetrachloride to induce chemical hepatitis followed 6 h later by oral saline administration; and the fourth group which

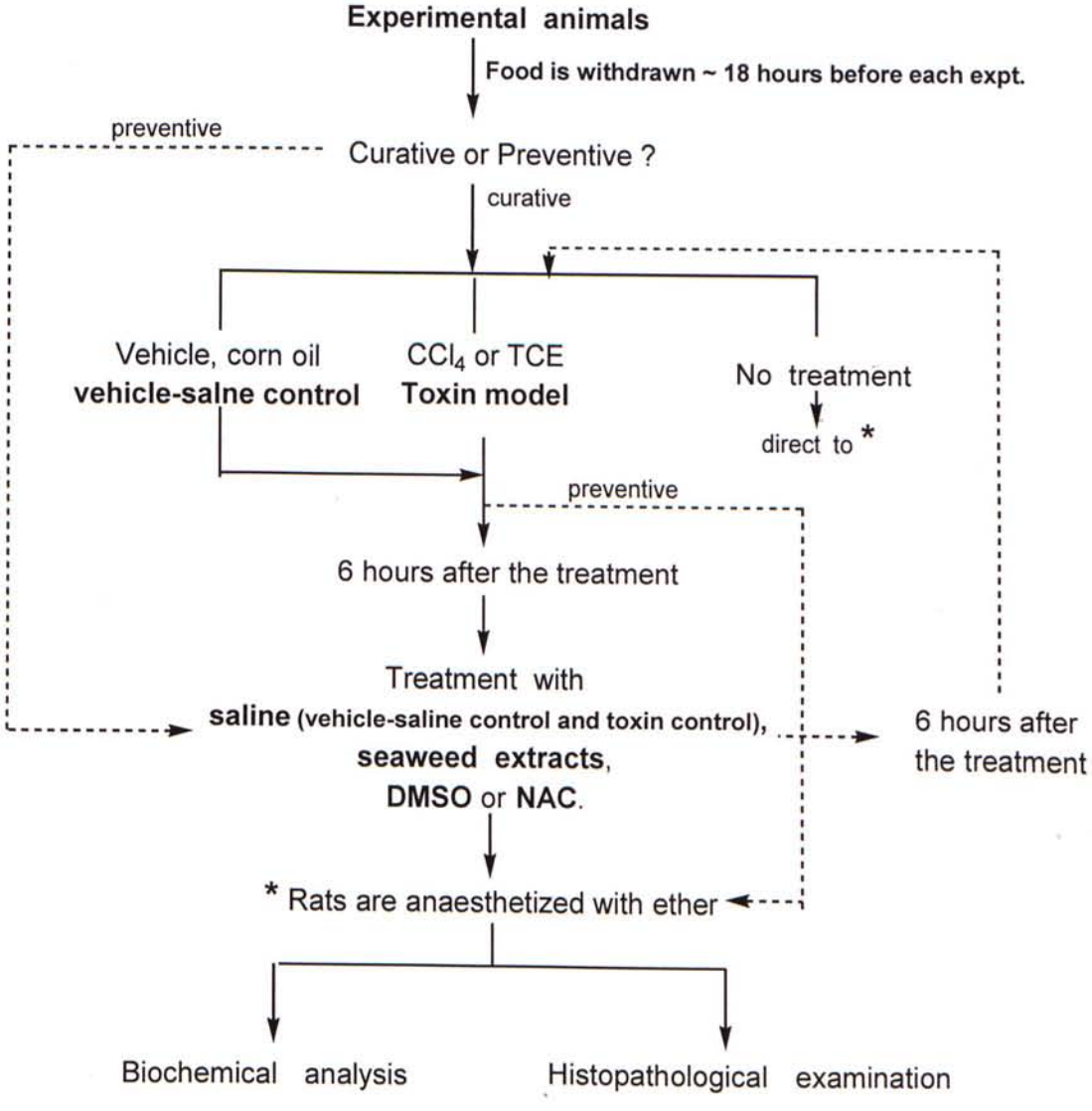


Fig. 3.7 The general experimental procedure of the project

was treated similarly to toxin control group except that each seaweed extract was individually administered instead of saline to evaluate their curative effects. Three dosages of seaweed extracts (150 mg/kg, 300 mg/kg and 600 mg/kg, dissolved in 10 ml saline) were used. Ten animals received one dosage of each seaweed extract. All treated animals were sacrificed 24 h after receiving the administration of the vehicle (corn oil) or hepatotoxin (CCl_4).

The preventive test was done in the same way as the curative test, except that the CCl_4 was administered 6 h before the treatment of saline and each seaweed extract. Seven rats were used in each treatment group. The CCl_4 (Merck, Germany) was used in the preventive test instead of CCl_4 (Ajax, Australia).

3.2.2.3 Biochemical assays.

The method used for determination of activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) followed that described in the section 3.2.1.3.

3.2.2.4 Organ weights.

The livers and kidneys of each treatment group were dissected out from the animals' bodies followed by the measurement of liver and kidney weight.

3.2.2.5 Histopathological examination.

(i) Light microscopy

A portion of the median lobe of the liver and left kidney were fixed in Bouin's fluid for 24 h and dehydrated though an ascending series of different percentage of ethanol; cleared in xylene. The liver and kidney specimens were then embedded in paraffin (melting point 56-58 °C), cut to 5-7 μm thickness by a microtome, and stained with Mayer's hematoxylin and eosin (H&E) for histopathological examination under light microscope. Detailed procedures for the histopathological processing were outlined in the Appendix B.

(ii) Electron microscopy

The livers from the second group of the curative test receiving CCl_4 and saline only were isolated and sliced into small pieces in cold 0.1 M phosphate buffer (pH 7.2). The samples were fixed in 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, with a final concentration of 2% paraformaldehyde added. The fixation was carried out at 4 °C for 2 h. The samples were then dehydrated in a series of ethanol (50%, 70%, 85%, 95% and three changes for absolute ethanol, 15 min for each change). The tissues were then infiltrated in a series of Spurr's epoxy resin medium at room tempeature, and embedded in pure Spurr's epoxy resin, polymerized at 68 °C in Reichert KT100 incubator for 18 h. The specimens were sectioned with Reichert Om2 ultracut using LKB KnifeMaker type 801B glass knife. The section was cut to 60 to 150 nm thickness. The thin sections were mounted on 200 mesh degreased copper grid. The sections were stained with 2% uranyl acetate in absolute ethanol and lead citrate for 30 min of each stain. The stained sections were examined under

Jeol JEM-1200 EX transmission electron microscope.

The same set of samples were studied with scanning electron microscopy. The fixation and dehydration procedures followed those in transmission electron microscopy except that they were carried out through the critical point drying process by LADD Research Industries' Critical Point Dryer (cat No: 28000128002) after dehydrated in absolute ethanol. The specimens were then coated with gold-palladium alloy by Edward's Sputter Coater S150B, and observed under Jeol JSM-5300 scanning electron microscope. The detailed procedures of reagent and tissue preparation for SEM and TEM were outlined in the Appendixes C to F.

3.2.2.6 Statistical analysis

For statistical analysis of the data, the Student's t-test and ANOVA were used to compare the levels of SGPT and SGOT in different treatments. All results are expressed in arithmetical means ± standard error of means. The data were regarded as significant and very significant if $p< 0.05$ and $p< 0.005$ respectively. A two-way ANOVA was used to assess the difference in the effect of different treatments and their concentrations on affecting the levels of SGPT and SGOT. Where significant differences have been detected, a Tukey HSD Test was used to identify the group(s) responsible for such differences.

3.2.3 Acute hepatotoxicity test of trichlororethylene in rats administered by oral and intraperitoneal routes.

3.2.3.1 Experimental protocol.

Trichloroethylene, which was used for studying the acute hepatotoxicity when taken dosage (1.25 ml/kg) the same as the CCl_4 treatment group in the section 3.2.2.2 for comparison. The different dosages of TCE (Sigma, U.S.A.) was made by dissolving in corn oil (Mazola, U.S.A.) and was administered intraperitoneally to the rats through the sterile disposable syringes equipped with hypodermic needles.

The experiments were divided into four sets, namely: **the TCE-1 (one-time oral group)** which received trichloroethylene (TCE) to study its acute hepatotoxicity. Five dosages of TCE-1 group (20%, 30% and 35% of TCE with 7 rats in each group; 40% TCE with 12 rats and 60% of TCE with 10 rats), which were given by gavage oral administration followed 6 h later by saline orally (10 ml/kg); the second set, namely: **the TCE-2 (two-time oral group)** which received TCE in four dosages with the experimental animals number (20% of TCE with 7 rats; 40% of TCE with 10 rats; and 50% and 60% of TCE with 5 rats in each group), which were given the first dosage of TCE in each dosage by gavage oral administration and 3 h later by second dosage of TCE, followed 6 h later from the first dosage, were given by saline orally (10 ml/kg). The second set was compared with no treatment group (10 rats) and vehicle-saline group (10 rats); the third set, namely **the TCE-3 (i.p. group)** which was treated similarly to that of **the TCE-1 group** except that intraperitoneal route was adopted instead of oral route. Six dosages of TCE-3 (i.p. group) with the experimental animals number (20% of TCE with 11 rats; 25% and 40% of TCE with

10 rats; and 50% and 60% of TCE with 5 rats in each group), which were given by i.p. administration followed 6 h later by saline intraperitoneally (10 ml/kg). The third set was compared with no treatment group (7 rats) and vehicle-saline control group (10 rats), which vehicle, corn oil (Mazole, U.S.A) was administered in i.p. route followed 6 h later by saline orally.

Based on the toxicity test performed above, the effective toxic dose of TCE was identified and a time course of this effective toxic dose was made to investigate the changes in the transaminase activity (SGPT and SGOT) in rats. Seven sets of time points with experimental animals number (6 h with 8 rats; 12 h with 7 rats; 27 h, 36 h, 48 h, 72 h and 102 h with 6 rats in each) which were given TCE as same as that of the effective toxic group followed 6 h later by saline orally (10 ml/kg).

3.2.3.2 Biochemical assays.

The method used for determination of transaminase activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) followed that described in the section 3.2.1.3. except that the serum was not diluted with saline.

3.2.3.3 Organ weights.

The livers and kidneys of each treatment group were dissected out from the bodies of the rats followed by the measurement of liver and kidney weight.

3.2.3.4 Histopathological examination.

(i) Light microscopy

A portion of the median lobe of the liver and left kidney of the TCE effective toxic dose group were investigated only. The method used in light microscopy was the same as described in section 3.2.1.5.

(ii) Electron microscopy

The livers from the TCE effective toxic dose group were isolated and sliced into small pieces in cold 0.1 M phosphate buffer (pH 7.2). The method used in electron microscopy was the same as described in section 3.2.2.5.

3.2.3.5 Statistical analysis.

For statistical analysis of the data, the Student's t-test was used to compare the levels of SGPT and SGOT in different treatments. All results are expressed in arithmetical mean ± standard error of means. The data were regarded as significance and very significance if $p<0.05$ and $p<0.005$ respectively.

3.2.4 Curative and Preventive tests of seaweed aqueous extracts against the TCE effective dose-induced toxicity.

3.2.4.1 Preparation of aqueous extracts of seaweeds.

Aqueous extracts of fresh samples of *Myagropsis myagroides* (S#3), *Sargassum henslowianum* (S#2), *S. siliquastrum* (S#4) and *Galaxaura* sp. (Gal) were used for the investigation. For extraction, the method used was the same as described in the section 3.2.1.1.

3.2.4.2 Experimental protocol (Fig. 3.7).

Trichlororethylene produces significant toxic effect when taken in suitable dosage (1.25 ml/kg). The 20% (v/v) TCE (Sigma, U.S.A.) was made by dissolving TCE in corn oil (Mazola, U.S.A.) and was administered intraperitoneally to the rats through the sterile disposable syringes equipped with hypodermic needles.

The experimental animals were divided into four groups, namely: the normal no treatment group (5 rats); the control group (5 rats) which received the vehicle (6.25 ml/kg, corn oil, Mazole, U.S.A.) by intraperitoneal route and normal saline (10 ml/kg) was also administered orally 6 h after the vehicle treatment; the third group (5 rats) which was treated similarly to group 2 except that 1.25 ml/kg of 20% (v/v) TCE was administered instead of vehicle; the fourth group which was treated similarly to group 3 except that each seaweed extract was individually administered instead of saline: A group of experimental animals (66 rats) which received TCE at effective toxic dose to induce toxic effect were followed 6 h later by seaweed extract to evaluate their curative effect. Three dosages of seaweed extracts (150 mg/kg, 300 mg/kg and 600 mg/kg, dissolved in 10 ml saline) except *Galaxaura* sp. extract (150 mg/kg and 300 mg/kg only, dissolved in 10 ml saline) were used. Six animals received one dosage of each seaweed extract. All treated animals were sacrificed 24 h after the administration of TCE. These animals were compared with the no

treatment group, vehicle-saline group and TCE effective toxic dose.

The preventive test was done in the same way as the curative test, except that the TCE was administered 6 h before the treatment of saline (5 rats) and each seaweed extract (6 rats in each treatment). The vehicle-saline group (control group, 5 rats) in the preventive test, which received normal saline (10 ml/kg) orally 6 h before the vehicle (6.25 ml/kg), corn oil (Mazole, U.S.A), which was administered intraperitoneally.

3.2.4.3 Biochemical assays.

The method used for determination of transaminases activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) followed that described in the section 3.2.1.3 except that the serum was not diluted with saline.

3.2.4.4 Organ weights.

The livers and kidneys of each treatment group were dissected out from the bodies of the rats followed by the measurement of liver and kidney weight.

3.2.4.5 Histopathological examination.

Light microscopy

A portion of the median lobe of the liver and left kidney of all treatment group was investigated. The method used in light microscopy was the same as that

described in the section 3.2.1.5.

3.2.5 Antidotal effects of dimethyl sulfoxide (DMSO) and N-acetylcysteine (NAC) against CCl_4- and TCE-induced poisoning in rats.

3.2.5.1 Experimental protocol (Fig. 3.7).

Carbon tetrachloride and trichloroethylene produce hepatotoxicity when taken in suitable dosage (1.25 ml/kg). The 20% (v/v) of CCl_4 (Sigma, U.S.A.) and TCE (Sigma, U.S.A.) were made by dissolving CCl_4 and TCE individually in corn oil (Mazola, U.S.A.) and were administered to the rats by gavage oral administration and intraperitoneal route respectively.

The preventive test was done in the same way as the curative test, except that the CCl_4 and TCE were administered 6 h before the treatment of saline, different dosages treatment of DMSO and NAC (7 rats in each CCl_4 treatment, 6 rats in each TCE treatment). The vehicle-saline group in the preventive test, which received normal saline (10 ml/kg) orally 6 h before the vehicle (6.25 ml/kg), corn oil (Mazole, U.S.A), which was administered orally and intraperitoneally in CCl_4 group and TCE group respectively.

The experimental animals were divided into four groups, namely: CCl_4 group and TCE group. The CCl_4 group (7 rats) which received carbon tetrachloride to induce chemical hepatitis followed 6 h later by oral saline administration; and the TCE group (6 rats) which received trichlororethylene to induce effective toxic effect followed 6 h later by oral saline administration. The third and the fourth group,

which were treated similarly to CCl_4 and TCE group except that dimethyl sulfoxide (DMSO) (Ajax, Australia) and N-acetylcysteine (NAC) (Sigma, U.S.A.) which were individually administered instead of saline to evaluate their curative effects. Three dosages of DMSO (25%, 50% and 75%, dissolved in saline) and NAC (150 mg/kg, 300 mg/kg and 600 mg/kg, dissolved in 10 ml saline) were used. Seven animals received one dosage of each DMSO and NAC dosages respectively by gavage oral administration. All treated animals were sacrificed 24 h after receiving the administration of CCl_4 or TCE.

3.2.5.2 Biochemical assays.

The method used for determination of transaminase activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) followed that described in the section 3.2.1.3 except that the serum was not diluted with saline in TCE group.

3.2.5.3 Organ weights.

The livers and kidneys of each treatment group were dissected out from the bodies of the rats followed by the measurement of liver and kidney weight.

3.2.5.4 Histopathological examination.

Light microscopy

A portion of the median lobe of the liver and left kidney of all treatment group was investigated. The method used in light microscopy was the same as described in section 3.2.1.5.

3.2.6 Hepatoprotective effect of seaweeds' methanol extract against CCl_4- and TCE-induced poisoning in rats.

3.2.6.1 Preparation of methanol extracts of seaweeds.

Methanol extracts of fresh samples of *Myagropsis myagroides* (S#3), *Sargassum henslowianum* (S#2), *S. siliquastrum* (S#4) and *Galaxaura* sp. (Gal) were used for the investigation. For extraction, washed seaweeds were weighed and blended with distilled water. They were kept at 4 °C for 1 day and then filtered through cotton gauze. The filtrates were centrifuged at 23,700 g (12000 rpm) for 20 min. After centrifugation, the pellets were freeze dried. The freeze dried pellets were place in the Soxhlet apparatus to reflux for 6 h by using pure methanol (Ajax, Australia). The total amount of methanol extract products were combined and evaporated to dryness under vacuum at 40 °C by using the rotor evaporator. The final residue was stored into airtight glass vials for aspiration under nitrogen gas to form dark-green viscous semisolid **(Fig 3.8)**. The final treated products were stored in the refrigerator until use.

3.2.6.2 Experimental protocol (Fig. 3.7).

Carbon tetrachloride and trichloroethylene produce hepatotoxicity when taken in suitable dosage (1.25 ml/kg). The 20% (v/v) of CCl_4 (Sigma, U.S.A.) and TCE (Sigma, U.S.A.) were made by dissolving CCl_4 and TCE individually in corn oil (Mazola, U.S.A.) and were administered to the rats by gavage oral administration and intraperitoneal route respectively. The 25% (v/v) of DMSO (Ajax, Australia) was made by dissolving it in 0.9% (v/v) saline and was administered to the rats by gavage oral administration.

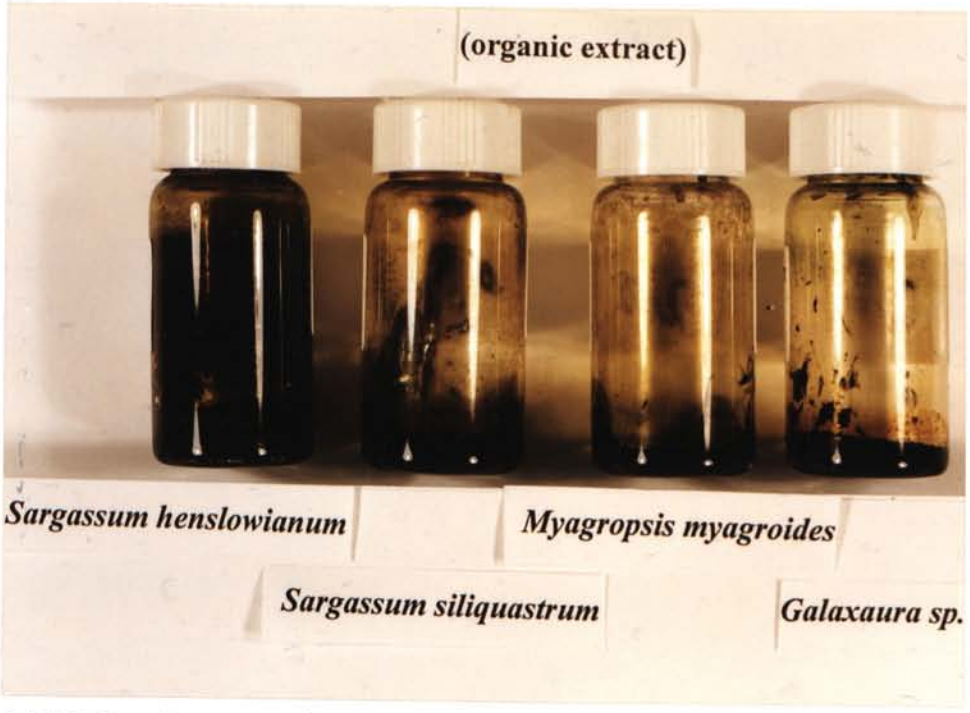


Fig. 3.8 Methanol extract of seaweed (*Sargassum henslowianum*; *S. siliquastrum*; *Myagropsis myagroides*; and *Galaxaura* sp., from left to right)

The experiment was divided into two sets, namely: CCl_4 group and TCE group. The two sets of experiment which were treated similarly to CCl_4 group and TCE group in the section 3.2.5.1 except that each seaweeds methanol extract was individually administered instead of saline 6 h post-treatment of toxins (CCl_4 and TCE) to evaluate their curative effects. The dosages of seaweed methanol extracts (300 mg/kg, dissolved in 25% DMSO) were used. Five animals received one dosage of each seaweed extract (*Myagropsis myagroides* (S#3), *Sargassum henslowianum* (S#2), *S. siliquastrum* (S#4)) for the two sets of experiment except the TCE group had one additional set of experiment which received extract of *Galaxaura* sp. (Gal). All treated animals were sacrificed 24 h after receiving the administration of the CCl_4 and TCE. These two sets of experiment were compared with the vehicle-saline group, no treatment group and 25% DMSO group in the section 3.2.5.1.

3.2.6.3 Biochemical assays.

The method used for determination of transaminase activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) followed that described in the section 3.2.1.3 except that the serum was not diluted with saline in TCE group.

3.2.6.4 Organ weights.

The livers and kidneys of each treatment group were dissected out from the bodies of the rats followed by the measurement of liver and kidney weight.

3.2.6.5 Histopathological examination.

Light microscopy

A portion of the median lobe of the liver and left kidney of all treatment group were investigated. The method used in light microscopy was the same as described in the section 3.2.1.5.

Chapter 4 RESULTS

4.1. Acute hepatotoxicity test on aqueous seaweed extracts.

4.1.1 The biochemical assays of the serum transaminase activity.

Table 3: Effect of seaweed extracts on transaminase activities (SGPT and SGOT) in rats

(Fig. 4.1, Fig. 4.2: p. 149 & p. 150 respectively)

	Treatment							
Parameter	No treatment	Control	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg
SGPT (IU/L)	12±0.5	11±0.7	12±0.6	11±0.7	12±0.8	11±0.1	11±0.8	12±0.5
SGOT (IU/L)	51±0.5	53±1.1	53±5.8	52±2.7	53±2.0	51±1.8	54±0.6	53±0.5

	Treatment							
Parameter	---	---	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg	Gal 150mg/kg	Gal 300mg/kg	---
SGPT (IU/L)	---	---	12±0.7	11±1.1	11±0.6	11±.0.5	11±0.9	---
SGOT (IU/L)	---	---	54±2.0	53±.2.2	53±1.7	51±1.5	51±1.6	---

Each value represents the mean±S.E.M. of 5 treated rats

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*, Gal= *Galaxaura* sp.

The values of SGPT and SGOT serum enzymes in all seaweed extract-treated group were comparable ($p>0.05$) with the values of the vehicle-saline control.

Comparison of the effect of different seaweed extracts.

Different concentrations of all three different seaweed extracts showed no significant differences in their ability to increase the levels of SGPT and SGOT ($p>0.05$, Two-way ANOVA). The three types of seaweed extracts: *S. henslowianum*, *M. myagroides*, *S. siliquastrum* and *Galaxaura* sp. have the equal effect on the SGPT and SGOT level ($p>0.05$, Turkey HSD test). Furthermore, increasing the concentration of seaweed extracts caused no any effect in the levels of both SGPT and SGOT ($p>0.05$, Tukey HSD test). There is no any significant different to be noted in the test ($p<0.05$).

4.1.2 The organ weight.

Table 4: Effect of seaweed extracts on organ weight (liver and kidney) in rats
(Fig. 4.3, Fig. 4.4: p. 151 & p. 152 respectively)

	Treatment							
Parameter	No treatment	Control	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg
liver weight (g/kg)	3.25×10^{-2} $\pm0.72\times10^{-3}$	3.32×10^{-2} $\pm0.67\times10^{-3}$	3.37×10^{-2} $\pm0.80\times10^{-3}$	3.32×10^{-2} $\pm1.41\times10^{-3}$	3.41×10^{-2} $\pm0.94\times10^{-3}$	3.49×10^{-2} $\pm0.93\times10^{-3}$	3.27×10^{-2} $\pm1.81\times10^{-3}$	3.37×10^{-2} $\pm0.92\times10^{-3}$
kidney weight (g/kg)	4.4×10^{-3} $\pm7.0\times10^{-5}$	4.2×10^{-3} $\pm4.9\times10^{-5}$	4.0×10^{-3} $\pm11\times10^{-5}$	4.1×10^{-3} $\pm9.5\times10^{-5}$	4.0×10^{-3} $\pm12\times10^{-5}$	4.2×10^{-3} $\pm9.1\times10^{-5}$	4.1×10^{-3} $\pm11\times10^{-5}$	4.1×10^{-3} $\pm8.9\times10^{-5}$

	Treatment							
Parameter	---	---	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg	Gal 150mg/kg	Gal 300mg/kg	---
liver weight (g/kg)	---	---	3.53×10^{-2} $\pm0.88\times10^{-3}$	3.34×10^{-2} $\pm1.48\times10^{-3}$	3.55×10^{-2} $\pm0.75\times10^{-3}$	3.29×10^{-2} $\pm0.86\times10^{-3}$	3.34×10^{-2} $\pm0.91\times10^{-3}$	---
kidney weight (g/kg)	---	---	4.2×10^{-3} $\pm13\times10^{-5}$	4.2×10^{-3} $\pm12\times10^{-5}$	4.0×10^{-3} $\pm15\times10^{-5}$	4.2×10^{-3} $\pm5.8\times10^{-5}$	4.2×10^{-3} $\pm8.7\times10^{-5}$	---

Each value represents the mean±S.E.M. (organ weight/body weight) of 5 treated rats

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*, Gal= *Galaxaura* sp.

The values of liver weight and kidney weight (g/kg) in all seaweed extract-treated group were comparable ($p>0.05$) with the values of the vehicle-saline control.

Comparison of the effect of different seaweed extracts.

Different concentrations of all three different seaweed extracts showed no significant differences in their ability to increase the acute increase of both liver and kidney weight ($p>0.05$, Two-way ANOVA). Furthermore, increasing the concentration of seaweed extracts caused no any effect in the values of both liver weight and kidney weight ($p>0.05$, Tukey HSD test). There is no any significant different to be noted in the test ($p<0.05$).

4.2 Curative and preventive tests of seaweed aqueous extracts against the CCl_4-induced hepatotoxicity.

4.2.1 The biochemical assays of the serum transaminase activity (Curative).

Table 5: Effect of seaweed extracts on CCl_4-induced elevation of SGPT and SGOT activities in rats (Curative)

(Fig. 4.5, Fig. 4.6: p. 153 & p. 154 respectively)

	Treatment					
Parameter	No treatment	Control	CCl_4 1.25ml/kg	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg
SGPT (IU/L)	11±0.5	12±0.6	1859 ±121	1402 ±47.4**	1113 ±60.1**	1021 ±78.1**
SGOT (IU/L)	51±0.8	54±1.9	2452 ±173	1723 ±60.1**	1450 ±36.0**	1151 ±97.1**

	Treatment					
Parameter	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg
SGPT (IU/L)	1274 ±132*	1115 ±19.8**	867 ±90.0**	1617 ±29.4	1414 ±46.5**	1433 ±52.8*
SGOT (IU/L)	1697 ±176*	1700 ±45.5**	1554 ±98.0**	1937 ±35.4*	1635 ±52.5**	1562 ±80.6**

Each value represents the mean±S.E.M. of 10 treated rats

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

Comparison of the effect of different seaweed extracts.

Different concentrations of all three different seaweed extracts showed significant differences in their ability to reduce the levels of SGPT and SGOT ($p<0.001$, Two-way ANOVA). Of these three types of seaweed extracts, those from *S. henslowianum* and *M. myagroides* appeared to be equally effective in reducing the levels of SGPT ($p>0.05$, Tukey HSD test) and those from *S. siliquastrum* were the least effective. Furthermore, the extracts from *S. henslowianum* were the most effective in reducing the levels of SGOT ($p<0.05$, Tukey HSD test), whereas those from *M. myogroides* and *S. siliquastrum* were not as effective. The effects from the latter two species were not significantly different ($p>0.05$, Tukey HSD test). Increasing the concentration of seaweed extracts was effective in reducing the levels of both SGPT and SGOT but only up to a point for SGPT. Increasing the concentration of extracts from 300 to 600 mg/kg for any one of the three seaweed extracts did not significantly increase their effect in reducing the SGPT levels ($p>0.05$, Tukey HSD test).

4.2.2 The organ weight. (Curative)

Table 6: Effect of seaweed extracts on CCl_4-induced increase of liver and kidney weights in rats (Curative)

(Fig. 4.7, Fig. 4.8: p. 155 & p. 156 respectively)

	Treatment					
Parameter	No treatment	Control	CCl_4 1.25ml/kg	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg
liver weight (g/kg)	3.23×10^{-2} $\pm0.68\times10^{-3}$	3.36×10^{-2} $\pm0.79\times10^{-3}$	5.36×10^{-2} $\pm1.04\times10^{-3}$	4.38×10^{-2} $\pm1.85\times10^{-3}$ *	4.36×10^{-2} $\pm1.85\times10^{-3}$ *	3.9×10^{-2} $\pm2.10\times10^{-3}$ **
kidney weight (g/kg)	4.4×10^{-3} $\pm7.4\times10^{-5}$	4.1×10^{-3} $\pm5.8\times10^{-5}$	4.5×10^{-3} $\pm9.1\times10^{-5}$	4.4×10^{-3} $\pm7.4\times10^{-5}$	4.4×10^{-3} $\pm5.8\times10^{-5}$	4.1×10^{-3} $\pm9.1\times10^{-5}$ **

	Treatment					
Parameter	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg
liver weight (g/kg)	4.86×10^{-2} $\pm1.69\times10^{-3}$ *	4.70×10^{-2} $\pm1.89\times10^{-3}$ **	4.56×10^{-2} $\pm0.72\times10^{-3}$ **	5.31×10^{-2} $\pm1.30\times10^{-3}$ *	5.00×10^{-2} $\pm1.30\times10^{-3}$	5.02×10^{-2} $\pm1.09\times10^{-3}$ *
kidney weight (g/kg)	4.3×10^{-3} $\pm1.1\times10^{-4}$	4.4×10^{-3} $\pm1.1\times10^{-4}$	4.4×10^{-3} $\pm7.63\times10^{-5}$	4.3×10^{-3} $\pm7.2\times10^{-5}$	4.3×10^{-3} $\pm6.8\times10^{-5}$	4.4×10^{-3} $\pm8.7\times10^{-5}$

Each value represents the mean±S.E.M. (organ weight/body weight) of 10 treated rats

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

Comparison of the effect of different seaweed extracts

Different concentrations of all three different seaweed extracts showed significant differences in their ability to reduce the acute increase of liver weight only ($p<0.001$, Two-way ANOVA). Of these three types of seaweed extracts, those from *S. henslowianum* appeared to be more effective in reducing the acute increase of liver weight than *M. myagroides* ($p<0.05$, Tukey HSD test) and those from *S. siliquastrum* were the least effective. Furthermore, the extracts from *S. henslowianum* (600 mg/kg) was effective in reducing the acute increase of kidney weight only ($p<0.05$, Tukey HSD test), whereas those from *S. henslowianum* (150 mg/kg and 300 mg/kg), *M. myogroides* and *S. siliquastrum* were not effective at all. The effects from the latter species group were not significantly different ($p>0.05$, Tukey HSD test). Increasing the concentration of seaweed extracts was effective in reducing the acute increase of liver weight and kidney weight but only up to a point for liver weight. Increasing the concentration of extracts from 300 to 600 mg/kg for any one of the three seaweed extracts did not significantly increase their effect in reducing the acute increase of liver weight except *S. henslowianum* ($p>0.05$, Tukey HSD test).

4.2.3 The biochemical assays of the serum transaminase activity. (Preventive)

Table 7: Effect of seaweed extracts on CCl_4-induced elevation of SGPT and SGOT activities in rats (Preventive)

(Fig. 4.9, Fig. 4.10: p. 157 & p. 158 respectively)

	Treatment					
Parameter	No treatment	Control	CCl_4 1.25ml/kg	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg
SGPT (IU/L)	10±0.2	12±0.2	1298 ±48	1294 ±84.6	1359 ±68.0	1208 ±95.5
SGOT (IU/L)	51±0.5	53±1.1	1864 ±65	1835 ±148	1853 ±76.9	1753 ±152

	Treatment					
Parameter	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg
SGPT (IU/L)	1096 ±64.9*	1014 ±42.4*	1043 ±55.6*	1296 ±66.4	1288 ±62.7*	1470 ±32.2*
SGOT (IU/L)	1844 ±220	1741 ±50.2	1717 ±125	1792 ±47.9	1860 ±48.2	1756 ±56.6

Each value represents the mean±S.E.M. of 7 treated rats

* Significantly different when compared with the toxin control, $p< 0.05$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

Comparison of the effect of different seaweed extracts.

Different concentrations of all three different seaweed extracts showed no significant differences in their ability to reduce the levels of SGPT and SGOT ($p>0.05$, Two-way ANOVA) except SGPT in all *M. myagroides* extracts treatment and 600 mg/kg *S. siliquastrum* extract treatment ($p<0.05$, Tukey HSD test). Of these three types of seaweed extracts, those from *S. henslowianum* and *S. siliquastrum* appeared to be equally effect in the levels of SGPT and SGOT ($p>0.05$, Tukey HSD test) except 600 mg/kg *S. siliquastrum* appeared to have the SGPT value higher than the toxin control ($p<0.05$, Tukey HSD test) and those from *M. myagroides* were the most effective in the reducing the levels of SGPT only. Furthermore, increasing the concentration of seaweed extracts was not effective in reducing the levels of both SGPT and SGOT. Increasing the concentration of extracts from 150 to 600 mg/kg for any one of the three seaweed extracts did not significantly affect the SGPT and SGOT levels ($p>0.05$, Tukey HSD test).

4.2.4 The organ weight. (Preventive)

Table 8: Effect of seaweed extracts on CCl_4-induced increase of liver and kidney weights in rats (Preventive)

(Fig. 4.11, Fig. 4.12: p. 159 & p. 160 respectively)

	Treatment					
Parameter	No treatment	Control	CCl_4 1.25ml/kg	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg
liver weight (g/kg)	3.29×10^{-2} $\pm0.75\times10^{-3}$	3.29×10^{-2} $\pm0.49\times10^{-3}$	4.91×10^{-2} $\pm1.47\times10^{-3}$	5.01×10^{-2} $\pm0.99\times10^{-3}$	4.68×10^{-2} $\pm1.25\times10^{-3}$	4.34×10^{-2} $\pm0.75\times10^{-3}$ *
kidney weight (g/kg)	4.1×10^{-3} $\pm4.0\times10^{-5}$	4.1×10^{-3} $\pm12\times10^{-5}$	4.7×10^{-3} $\pm9.0\times10^{-5}$	4.3×10^{-3} $\pm15\times10^{-4}$ *	4.2×10^{-3} $\pm1.1\times10^{-4}$ *	4.2×10^{-3} $\pm1.1\times10^{-4}$ *

	Treatment					
Parameter	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg
liver weight (g/kg)	4.57×10^{-2} $\pm0.75\times10^{-3}$	4.28×10^{-2} $\pm1.10\times10^{-3}$ *	4.41×10^{-2} $\pm1.25\times10^{-3}$ **	4.94×10^{-2} $\pm1.04\times10^{-3}$	4.98×10^{-2} $\pm2.01\times10^{-3}$	4.96×10^{-2} $\pm1.76\times10^{-3}$
kidney weight (g/kg)	4.4×10^{-3} $\pm1.5\times10^{-4}$ *	4.1×10^{-3} $\pm0.6\times10^{-4}$ **	4.1×10^{-3} $\pm0.3\times10^{-4}$ **	4.4×10^{-3} $\pm5.0\times10^{-5}$ *	4.4×10^{-3} $\pm9.0\times10^{-5}$ **	4.1×10^{-3} $\pm11\times10^{-5}$ **

Each value represents the mean±S.E.M. (organ weight/body weight) of 7 treated rats

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

Comparison of the effect of different seaweed extracts.

Different concentrations of all three different seaweed extracts showed significant differences in their ability to reduce the acute increase of kidney weight only ($p<0.001$, Two-way ANOVA). Of these three types of seaweed extracts, those from *S. henslowianum* and *M. myagroides* appeared to be equally effective in reducing the acute increase of kidney weight ($p>0.05$, Tukey HSD test) and those from *S. siliquastrum* were the least effective besides the 600 mg/kg of those extracts were applied. Furthermore, the extracts from *S. henslowianum* (600 mg/kg) and *M. myagroides* (300 and 600 mg/kg) were the most effective in reducing the acute increase of liver weight ($p<0.05$, Tukey HSD test), whereas those from *S. siliquastrum* were not as effective at all in reducing the acute increase of liver weight. The effects from the latter species were not significantly different by their different treatment applied ($p>0.05$, Tukey HSD test). Increasing the concentration of seaweed extracts was not effective in reducing the acute increase of both liver and kidney weight. But, increasing the concentration of extracts from 300 to 600 mg/kg for any one of the three seaweed extracts did not significantly increase their effect in reducing the acute increase of liver ($p>0.05$, Tukey HSD test) besides the extracts from *S. henslowianum* ($p<0.05$, Tukey HSD test).

4.3 Acute hepatotoxicity test of trichlororethylene in rats administered by oral and intraperitoneal routes.

4.3.1 Oral route.

4.3.1.1 One-time oral route.

(Table 9. Fig. 4.13, Fig. 4.14 & Fig. 4.17: p. 68, p.161, p. 162 & p. 165 respectively)

Based on the table shown, significant difference ($p<0.05$) can be noted in transaminase activities and organ weight from the 40% to 60% TCE applied. The death of experimental animals was also noted in those dosages.

4.3.1.2 Two-time oral route.

(Table 10, Fig. 4.15, Fig. 4.16 & Fig. 4.18: p. 69, p. 163, p164 & p. 166 respectively)

Based on the table shown, significant difference ($p<0.05$) can be noted in transaminase activities and organ weight at the 40% TCE applied. The death of experimental animals was also noted in that dosages. 100% of mortality rate was noted at 50% and 60% TCE applied.

4.3.2 Intraperitoneal route.

(Table 11, Fig. 4.19, Fig. 4.20 & Fig. 4.21: p. 70, p. 167, p. 168 & p. 169

respectively)

Based on the table shown, significant difference ($p<0.05$) can be noted in transaminase activities at the 20% t TCE applied onwards. No significant difference can be noted in the liver and kidney weights except the 40% TCE was applied ($p<0.05$, kidney weight). The death of experimental animals was also noted in the dosages of 25% to 60% TCE. 100% of mortality rate was noted at 50% and 60% TCE applied. Only 20% TCE in i.p. can induce the elevated levels of SGPT and SGOT significantly ($p<0.05$) with the 100% survival rate obtained when compared with the vehicle-saline control. However, there is no significant difference in liver and kidney weights when compared with those from the vehicle-saline control. **(20% TCE in i.p. is regarded as effective dose)**

4.3.3 Time course of the effective dose of 20% TCE in i.p. route.

(Table 12 & Fig. 4.22 to Fig. 4.25: p. 71, p. 170 to p. 173 respectively)

Based on the table shown, significant difference ($p<0.05$) can be noted in transaminase activities in all time except 36 h to 102 h of SGPT levels. There is a trend of SGPT and SGOT levels to return to normal. No significant difference can be noted in the liver and kidney weights except the 6 h ($p<0.05$). There are increase and decrease in size of liver and kidney respectively.

Table 9: Change of transaminase activities (SGPT and SGOT) and organ weight (liver and kidney) after a single oral dosage of TCE in different percentage.

Group	Dosage (%), 1.25 ml/kg	No. of animals used	No. of survivors	survival rate (%)	SGPT (IU/L)	SGOT (IU/L)	Liver weight (g/kg)	Kidney weight (g/kg)
no treatment	---	**10**	**10**	**100**	12±0.8	51±1.2	3.29×10^{-2} $\pm0.88\times10^{-3}$	4.2×10^{-3} $\pm6.8\times10^{-5}$
vehicle-saline	---	**10**	**10**	**100**	13±0.8	54±1.5	3.33×10^{-2} $\pm0.89\times10^{-3}$	4.1×10^{-3} $\pm6.4\times10^{-5}$
TCE 20%	**20**	**7**	**7**	**100**	12±0.6	55±3.3	3.65×10^{-2} $\pm0.80\times10^{-3}$	4.1×10^{-3} $\pm6.7\times10^{-5}$
TCE 30%	**30**	**7**	**7**	**100**	11±0.7	55±5.0	3.81×10^{-2} $\pm0.58\times10^{-3}$ **	3.8×10^{-3} $\pm15\times10^{-5}$
TCE 35%	**35**	**7**	**7**	**100**	11±0.6	51±3.1	3.82×10^{-2} $\pm0.15\times10^{-3}$ **	3.9×10^{-3} $\pm4.0\times10^{-5}$ *
TCE 40%	**40**	**30**	**12**	**40**	20±0.8 **	86±3.1 **	3.60×10^{-2} $\pm0.59\times10^{-3}$ **	3.9×10^{-3} $\pm5.3\times10^{-5}$ **
TCE 60%	**60**	**10**	**3**	**30**	26±4.0 **	134±16 **	5.14×10^{-2} $\pm1.30\times10^{-3}$ **	5.0×10^{-3} $\pm17\times10^{-5}$ **

* Significantly different when compared with the vehicle-saline control, $p< 0.05$.

** Significantly different when compared with the vehicle-saline control, $p< 0.005$.

Table 10: Change of transaminase activities (SGPT and SGOT) and organ weight (liver and kidney) after a twice oral dosage of TCE in different percentage.

Group	Dosage (%), 1.25 ml/kg	No. of animals used	No. of survivors	survival rate (%)	SGPT (IU/L)	SGOT (IU/L)	Liver weight (g/kg)	Kidney weight (g/kg)
no treatment	---	**10**	**10**	**100**	11±0.7	50±0.5	3.21×10^{-2} $\pm0.56\times10^{-3}$	4.4×10^{-3} $\pm6.7\times10^{-5}$
vehicle-saline	---	**10**	**10**	**100**	10±0.8	52±1.1	3.28×10^{-2} $\pm0.89\times10^{-3}$	4.3×10^{-3} $\pm5.0\times10^{-5}$
TCE 20%	**20**	7	7	**100**	14±1.7	64±3.6 *	4.16×10^{-2} $\pm0.56\times10^{-3}$ **	4.1×10^{-3} $\pm9.7\times10^{-5}$
TCE 40%	**40**	**10**	**4**	**40**	18±2.4 *	71±3.9 **	4.66×10^{-2} $\pm2.72\times10^{-3}$ **	4.8×10^{-3} $\pm5.2\times10^{-5}$ *
TCE 50%	**50**	**5**	**0**	**0**	—	—	—	—
TCE 60%	**60**	**5**	**0**	**0**	—	—	—	—

* Significantly different when compared with the vehicle-saline control, $p< 0.05$.

** Significantly different when compared with the vehicle-saline control, $p< 0.005$.

Table 11: Change of transaminase activities (SGPT and SGOT) and organ weight (liver and kidney) after a i.p. dosage of TCE in different percentage.

Group	Dosage (%), 1.25 ml/kg	No. of animals used	No. of survivors	survival rate (%)	SGPT (IU/L)	SGOT (IU/L)	Liver weight (g/kg)	Kidney weight (g/kg)
no treatment	---	7	7	100	11±0.5	51±0.8	3.23×10^{-2} $\pm0.68\times10^{-3}$	4.4×10^{-3} $\pm7.4\times10^{-5}$
vehicle-saline	---	10	10	100	12±0.3	51±0.7	3.36×10^{-2} $\pm0.79\times10^{-3}$	4.1×10^{-3} $\pm5.8\times10^{-5}$
TCE 20%	20	11	11	100	16±1.1 *	150±8.5 **	3.38×10^{-2} $\pm0.87\times10^{-3}$	4.0×10^{-3} $\pm7.6\times10^{-5}$
TCE 25%	25	10	8	80	45±5.7 **	200±9.9 **	3.44×10^{-2} $\pm1.03\times10^{-3}$	4.1×10^{-3} $\pm11\times10^{-5}$
TCE 30%	30	10	8	80	106±2.1 **	222±5.6 **	3.20×10^{-2} $\pm0.59\times10^{-3}$	4.1×10^{-3} $\pm7.0\times10^{-5}$
TCE 40%	40	10	3	30	103±8.9 **	231±12 **	3.10×10^{-2} $\pm1.55\times10^{-3}$	3.5×10^{-3} $\pm11\times10^{-5}$ **
TCE 50%	50	5	0	0	—	—	—	—
TCE 60%	60	5	0	0	—	—	—	—

* Significantly different when compared with the vehicle-saline control, $p< 0.05$.

** Significantly different when compared with the vehicle-saline control, $p< 0.005$.

Table 12: Time course of transaminase activities (SGPT and SGOT) and organ weight (liver and kidney) after a i.p. dosage of 20% TCE in rats.

Group	Time (h)	No. of animals used	SGPT (IU/L)	SGOT (IU/L)	Liver weight (g/kg)	Kidney weight (g/kg)
vehicle-saline	**regarded as 0 h**	**7**	12±0.5	53±0.8	3.33×10^{-2} $\pm0.72\times10^{-3}$	3.9×10^{-3} $\pm4.8\times10^{-5}$
TCE 20%	**6**	**8**	32±1.7 **	170±3.7 **	3.51×10^{-2} $\pm0.76\times10^{-3}$ *	3.9×10^{-3} $\pm5.4\times10^{-5}$ *
TCE 20%	**12**	**7**	30±1.9 **	180±5.0 **	3.36×10^{-2} $\pm0.60\times10^{-3}$	4.1×10^{-3} $\pm11\times10^{-5}$
TCE 20%	**24**	**11**	15±0.7 *	152±6.3 **	3.38×10^{-2} $\pm0.87\times10^{-3}$	4.0×10^{-3} $\pm7.6\times10^{-5}$
TCE 20%	**27**	**6**	15±0.4 *	81.4±7.8 **	3.31×10^{-2} $\pm0.53\times10^{-3}$	4.1×10^{-3} $\pm13\times10^{-5}$
TCE 20%	**36**	**6**	12±0.7	85.5±4.9 **	3.37×10^{-2} $\pm1.14\times10^{-3}$	4.1×10^{-3} $\pm10\times10^{-5}$
TCE 20%	**48**	**6**	12±1.3	68.5±5.0 **	3.40×10^{-2} $\pm1.12\times10^{-3}$	4.1×10^{-3} $\pm8.2\times10^{-5}$
TCE 20%	**72**	**6**	12±1.1	58.4±4.1 *	3.29×10^{-2} $\pm1.00\times10^{-3}$	4.1×10^{-3} $\pm12\times10^{-5}$
TCE 20%	**102**	**6**	13±1.2	58.5±1.7 **	3.32×10^{-2} $\pm1.17\times10^{-3}$	4.1×10^{-3} $\pm11\times10^{-5}$

* Significantly different when compared with the vehicle-saline control, $p< 0.05$.

** Significantly different when compared with the vehicle-saline control, $p< 0.005$.

4.4 Curative and preventive tests of seaweeds aqueous crude extracts against the TCE effective dose-induced toxicity.

4.4.1 The biochemical assays of the serum transaminase activity (Curative).

Table 13: Effect of seaweed extracts on TCE-induced elevation of SGPT and SGOT activities in rats (Curative)

(Fig. 4.26, Fig. 4.27: p. 174 & p. 175 respectively)

	Treatment							
Parameter	No treatment	Control	TCE 1.25ml/kg	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg	---	---
SGPT (IU/L)	11±0.5	11±0.8	14.25 ±1.3	11.32 ±0.89*	11.56 ±1.09*	11.22 ±1.08*	---	---
SGOT (IU/L)	52±1.2	55±1.4	153.4 ±6.4	111.0 ±16.9*	116.5 ±2.80**	85.25 ±4.36**	---	---

	Treatment							
Parameter	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg	Gal 150mg/kg	Gal 300mg/kg
SGPT (IU/L)	13.15 ±0.91*	13.40 ±0.85*	10.61 ±0.96**	15.34 ±1.28	12.40 ±0.76*	11.47 ±0.85*	13.24 ±0.76*	10.79 ±1.08**
SGOT (IU/L)	101.4 ±6.95**	100.0 ±5.42**	87.94 ±1.45**	130.5 ±6.64	128.1 ±4.83*	126.5 ±8.11*	125.8 ±4.48*	92.57 ±2.07**

Each value represents the mean±S.E.M. of 5 treated rats except seaweed extract-treated groups which have 6 treated rats in each group.

* Significantly different when compared with the toxin control, $p < 0.05$.

** Significantly different when compared with the toxin control, $p < 0.005$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

Comparison of the effect of different seaweed extracts.

Different concentrations of all three different seaweed extracts showed significant differences in their ability to reduce the levels of SGPT and SGOT ($p<0.001$, Two-way ANOVA). Of these four types of seaweed extracts, those from *Galaxaura* sp. could be the most effective than other species at their corresponding concentration ($p<0.05$, Turkey HSD test). *S. henslowianum* and *M. myagroides* appeared to be equally effective in reducing the levels of SGOT and SGPT (only at concentration of 600 mg/kg) ($p>0.05$, Tukey HSD test) and those from *S. siliquastrum* were the least effective. Furthermore, the extract from *Galaxaura* sp. (600 mg/kg) was the most effective in reducing the levels of SGPT and SGOT ($p<0.05$, Tukey HSD test), whereas those from *S. henslowianum* and *M. myagroides* were not as effective at that concentration. In general, increasing the concentration of seaweed extracts was effective in reducing the levels of both SGPT and SGOT except those from the SGOT of *S. siliquastrum*. Increasing the concentration of extracts from 300 to 600 mg/kg for any one of the three seaweed extracts (brown seaweeds) did significantly increase their effect in reducing the SGPT and SGOT levels ($p<0.05$, Tukey HSD test) except those from the SGPT level of. *S. henslowianum* and SGOT level of *S. siliquastrum* ($p>0.05$, Turkey HSD test). Moreover, increasing the concentration of extracts from 150 to 300 mg/kg of *Galaxaura* sp. did significantly increase the effect in reducing the SGPT and SGOT levels ($p<0.05$, Turkey HSD test).

4.4.2 The organ weight. (Curative)

Table 14: Effect of seaweed extracts on liver and kidney weights in TCE-treated rats (Curative)

(Fig. 4.28, Fig. 4.29: p. 176 & p. 177 respectively)

	Treatment							
Parameter	No treatment	Control	TCE 1.25ml/kg	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg	---	---
liver weight (g/kg)	3.33×10^{-2} $\pm0.76\times10^{-3}$	3.35×10^{-2} $\pm0.89\times10^{-3}$	3.36×10^{-2} $\pm0.84\times10^{-3}$	3.42×10^{-2} $\pm0.95\times10^{-3}$	3.48×10^{-2} $\pm0.40\times10^{-3}$	3.4×10^{-2} $\pm0.56\times10^{-3}$	---	---
kidney weight (g/kg)	4.2×10^{-3} $\pm6.3\times10^{-5}$	4.3×10^{-3} $\pm7.8\times10^{-5}$	4.1×10^{-3} $\pm7.3\times10^{-5}$	3.9×10^{-3} $\pm7.4\times10^{-5}$	4.2×10^{-3} $\pm8.1\times10^{-5}$	4.1×10^{-3} $\pm6.8\times10^{-5}$	---	---

	Treatment							
Parameter	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg	Gal 150mg/kg	Gal 300mg/kg
liver weight (g/kg)	3.46×10^{-2} $\pm0.90\times10^{-3}$	3.46×10^{-2} $\pm0.73\times10^{-3}$	3.45×10^{-2} $\pm0.54\times10^{-3}$	3.61×10^{-2} $\pm0.50\times10^{-3}$ *	3.73×10^{-2} $\pm0.96\times10^{-3}$ *	3.61×10^{-2} $\pm0.46\times10^{-3}$ *	3.51×10^{-2} $\pm0.42\times10^{-3}$	3.56×10^{-2} $\pm0.80\times10^{-3}$
kidney weight (g/kg)	4.1×10^{-3} $\pm8.5\times10^{-5}$	4.2×10^{-3} $\pm7.8\times10^{-5}$	3.9×10^{-3} $\pm12.0\times10^{-5}$	4.3×10^{-3} $\pm8.3\times10^{-5}$ *	4.5×10^{-3} $\pm4.6\times10^{-5}$ **	4.4×10^{-3} $\pm9.28\times10^{-5}$ *	4.1×10^{-3} $\pm9.5\times10^{-5}$	4.0×10^{-3} $\pm9.3\times10^{-5}$

Each value represents the mean±S.E.M. (organ weight/body weight) of 5 treated rats except seaweed extract-treated groups which have 6 treated rats in each group.

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

Comparison of the effect of different seaweed extracts.

Different concentrations of all four different seaweed extracts showed no significant differences with the liver and kidney weights of toxin control ($p>0.05$, Two-way ANOVA) except *S. siliquastrum*. Of these four types of seaweed extracts, those from *S. siliquastrum* appeared to generate toxic effect in increase of liver and kidney weights ($p<0.05$, Tukey HSD test). Increasing the concentration of seaweed extracts was not effective in changing values of liver weight and kidney weight ($p>0.05$, Turkey HSD test). Increasing the concentration of extracts from 150 to 600 mg/kg for any one of the three brown seaweed extracts and 150 to 300 mg/kg for the red seaweed extracts did not significantly increase their effect in any change of liver and kidney weights ($p>0.05$, Tukey HSD test).

4.4.3 The biochemical assays of the serum transaminase activity. (Preventive)

Table 15: Effect of seaweed extracts on TCE-induced elevation of SGPT and SGOT activities in rats (Preventive)

(Fig. 4.30, Fig. 4.31: p. 178 & p. 179 respectively)

	Treatment							
Parameter	No treatment	Control	TCE 1.25ml/kg	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg	---	---
SGPT (IU/L)	11±0.4	12±0.6	16 ±3.1	14.90 ±0.96	15.24 ±1.10	15.26 ±0.90	---	---
SGOT (IU/L)	51±1.2	49±3.0	152 ±5.0	103.0 ±2.50**	104.9 ±4.90**	103.3 ±3.70**	---	---

	Treatment							
Parameter	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg	Gal 150mg/kg	Gal 300mg/kg
SGPT (IU/L)	15.43 ±1.28	15.91 ±1.51	15.24 ±0.85	12.98 ±0.51*	12.59 ±1.15*	12.16 ±0.85*	14.44 ±0.99	12.45 ±0.90*
SGOT (IU/L)	117.6 ±5.81**	117.0 ±4.41**	117.1 ±4.24**	131.2 ±8.37*	116.8 ±2.28**	108.9 ±5.36**	110.5 ±6.24**	83.60 ±12.4**

Each value represents the mean±S.E.M. of 5 treated rats except TCE toxin control and seaweed extract-treated groups which have 6 treated rats in each group.

* Significantly different when compared with the toxin control, $p < 0.05$.

** Significantly different when compared with the toxin control, $p < 0.005$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

Comparison of the effect of different seaweed extracts.

Different concentrations of all four different seaweed extracts showed significant differences in their ability to reduce the levels of SGPT and SGOT ($p<0.05$, Two-way ANOVA) except SGPT in all *S. henslowianum* and *M. myagroides* extracts treatments ($p>0.05$, Tukey HSD test). Of these three types of seaweed extracts, those from *S. henslowianum* and *M. myagroides* appeared to be equally effect in the levels of SGPT ($p>0.05$, Tukey HSD test). Extracts from *S. siliquastrum* were the most effective in the reducing the levels of SGPT only ($p<0.05$, Turkey HSD test). Furthermore, increasing the concentration of seaweed extracts was not effective in reducing the levels of both SGPT and SGOT except *Galaxaura* sp. extracts ($p<0.05$, Turkey HSD test). Increasing the concentration of extracts from 150 to 600 mg/kg for any one of the three seaweed extracts did not significantly effect in the SGPT and SGOT levels ($p>0.05$, Tukey HSD test) except *S. siliquastrum* ($p<0.05$, Turkey HSD test).

4.4.4 The organ weight. (Preventive)

Table 16: Effect of seaweed extracts on liver and kidney weights in TCE-treated rats (Preventive)

(Fig. 4.32, Fig. 4.33: p. 180 & p. 181 respectively)

	Treatment							
Parameter	No treatment	Control	TCE 1.25ml/kg	S#2 150mg/kg	S#2 300mg/kg	S#2 600mg/kg	---	---
liver weight (g/kg)	3.31×10^{-2} $\pm0.62\times10^{-3}$	3.31×10^{-2} $\pm0.84\times10^{-3}$	3.30×10^{-2} $\pm0.81\times10^{-3}$	3.38×10^{-2} $\pm1.50\times10^{-3}$	3.34×10^{-2} $\pm1.10\times10^{-3}$	3.38×10^{-2} $\pm1.07\times10^{-3}$	---	---
kidney weight (g/kg)	4.1×10^{-3} $\pm6.7\times10^{-5}$	4.2×10^{-3} $\pm12\times10^{-5}$	4.2×10^{-3} $\pm7.5\times10^{-5}$	4.2×10^{-3} $\pm11\times10^{-5}$	4.2×10^{-3} $\pm12\times10^{-5}$	4.2×10^{-3} $\pm7.5\times10^{-5}$	---	---

	Treatment							
Parameter	S#3 150mg/kg	S#3 300mg/kg	S#3 600mg/kg	S#4 150mg/kg	S#4 300mg/kg	S#4 600mg/kg	Gal 150mg/kg	Gal 300mg/kg
liver weight (g/kg)	3.32×10^{-2} $\pm0.39\times10^{-3}$	3.24×10^{-2} $\pm1.29\times10^{-3}$	3.24×10^{-2} $\pm0.77\times10^{-3}$	3.56×10^{-2} $\pm0.70\times10^{-3}$ *	3.53×10^{-2} $\pm0.73\times10^{-3}$ *	3.81×10^{-2} $\pm1.06\times10^{-3}$ **	3.39×10^{-2} $\pm1.34\times10^{-3}$	3.42×10^{-2} $\pm0.27\times10^{-3}$
kidney weight (g/kg)	4.1×10^{-3} $\pm14\times10^{-5}$	4.3×10^{-3} $\pm13\times10^{-5}$	4.2×10^{-3} $\pm12\times10^{-5}$	4.1×10^{-3} $\pm11\times10^{-5}$	4.3×10^{-3} $\pm15\times10^{-5}$	4.2×10^{-3} $\pm8.7\times10^{-5}$	4.3×10^{-3} $\pm14\times10^{-5}$	4.2×10^{-3} $\pm7.5\times10^{-5}$

Each value represents the mean±S.E.M. (organ weight/body weight) of 5 treated rats except TCE toxin control and seaweed extract-treated groups which have 6 treated rats in each group.

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

Comparison of the effect of different seaweed extracts.

Different concentrations of all four different seaweed extracts showed no significant differences in their ability to cause any effect of liver and kidney weights ($p>0.05$, Two-way ANOVA) except increase of liver weights with the *S. siliquastrum* extracts applied ($p<0.05$, Turkey HSD test). Of these four types of seaweed extracts, all appeared to be equally effective in the effect of liver and kidney weights ($p>0.05$, Tukey HSD test) besides those from *S. siliquastrum* were the most effective in causing the increase of liver and kidney weights. Increasing the concentration of seaweed extracts was not effective in causing the effect of both liver and kidney weight. But, increasing the concentration of extracts from 300 to 600 mg/kg for *S. siliquastrum* extracts significantly increase their effect in the increase of liver weight ($p<0.05$, Tukey HSD test).

4.5 Antidotal effects of dimethyl sulfoxide (DMSO) and N-acetylcysteine (NAC) against CCl_4- and TCE-induced poisoning in rats.

4.5.1 The biochemical assays of the serum transaminase activity (Curative).

Table 17: Effect of DMSO and NAC on CCl_4-induced elevation of SGPT and SGOT activities in rats (Curative)

(Fig. 4.34, Fig. 4.35, Fig. 4.36 & Fig. 4.37: p. 182, p. 183 p. 184 & p. 185 respectively)

			Treatment 1			
Parameter	No treatment	Control	CCl_4 1.25ml/kg	DMSO 25%	DMSO 50%	DMSO 75%
SGPT (IU/L)	12±0.7	12±0.5	1255 ±107	1252 ±121	343.9 ±60.7**	287.4 ±23.7**
SGOT (IU/L)	48±1.8	51±1.7	2118 ±96.0	2143 ±95.3	1361 ±94.8**	1355 ±67.9**

			Treatment 2			
Parameter	No treatment	Control	CCl_4 1.25ml/kg	NAC 150 mg/kg	NAC 300 mg/kg	NAC 600 mg/kg
SGPT (IU/L)	11±1.1	10±0.7	1235 ±97.0	1219 ±123	975.2 ±156	305.0 ±66.8**
SGOT (IU/L)	52±1.4	51±1.0	2097 ±96.0	1885 ±97.2	1780 ±72.9**	1227 ±102**

Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group.

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Table 18: Effect of DMSO and NAC on TCE-induced elevation of SGPT and SGOT activities in rats (Curative)

(Fig. 4.38, Fig. 4.39, Fig. 4.40 & Fig. 4.41: p. 186, p. 187, p. 188 & p. 189 respectively)

			Treatment 1			
Parameter	No treatment	Control	TCE 1.25ml/kg	DMSO 25%	DMSO 50%	DMSO 75%
SGPT (IU/L)	13±0.7	12±0.5	16.06 ±1.10	15.58 ±1.54	11.88 ±0.90*	11.94 ±0.89*
SGOT (IU/L)	52±1.1	50±1.2	150.2 ±8.52	140.1 ±2.54	108.3 ±6.96**	109.6 ±4.28**

			Treatment 2			
Parameter	No treatment	Control	TCE 1.25ml/kg	NAC 150 mg/kg	NAC 300 mg/kg	NAC 600 mg/kg
SGPT (IU/L)	11±0.9	13±0.7	16.46 ±1.14	14.19 ±0.98	13.22 ±1.29	13.5 ±1.31
SGOT (IU/L)	53±0.5	51±1.0	151.3 ±8.52	132.6 ±7.74	99.13 ±6.68**	85.38 ±6.07**

Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group.

* Significantly different when compared with the toxin control, $p < 0.05$.

** Significantly different when compared with the toxin control, $p < 0.005$.

4.5.2 The organ weight. (Curative)

Table 19: Effect of DMSO and NAC on CCl_4-induced increase of liver and kidney weights in rats (Curative)

(Fig. 4.42, Fig. 4.43, Fig. 4.44 & Fig. 4.45: p. 190, p. 191 p. 192 & p. 193 respectively)

	Treatment 1					
Parameter	No treatment	Control	CCl_4 1.25ml/kg	DMSO 25%	DMSO 50%	DMSO 75%
liver weight (g/kg)	3.28×10^{-2} $\pm0.65\times10^{-3}$	3.34×10^{-2} $\pm0.70\times10^{-3}$	5.36×10^{-2} $\pm1.04\times10^{-3}$	4.95×10^{-2} $\pm2.15\times10^{-3}$	4.92×10^{-2} $\pm1.46\times10^{-3}$	4.29×10^{-2} $\pm1.08\times10^{-3}$ **
kidney weight (g/kg)	4.3×10^{-3} $\pm6.6\times10^{-5}$	4.2×10^{-3} $\pm5.2\times10^{-5}$	4.5×10^{-3} $\pm9.1\times10^{-5}$	4.4×10^{-3} $\pm13\times10^{-5}$	4.4×10^{-3} $\pm11\times10^{-5}$	4.4×10^{-3} $\pm8.8\times10^{-5}$

	Treatment 2					
Parameter	No treatment	Control	CCl_4 1.25ml/kg	NAC 150 mg/kg	NAC 300 mg/kg	NAC 600 mg/kg
liver weight (g/kg)	3.30×10^{-2} $\pm0.59\times10^{-3}$	3.33×10^{-2} $\pm0.67\times10^{-3}$	5.13×10^{-2} $\pm0.98\times10^{-3}$	4.86×10^{-2} $\pm0.93\times10^{-3}$	4.89×10^{-2} $\pm1.21\times10^{-3}$	4.44×10^{-2} $\pm0.88\times10^{-3}$ *
kidney weight (g/kg)	4.2×10^{-3} $\pm5.9\times10^{-5}$	4.1×10^{-3} $\pm5.0\times10^{-5}$	4.5×10^{-3} $\pm8.7\times10^{-5}$	4.5×10^{-3} $\pm8.2\times10^{-5}$	4.3×10^{-3} $\pm9.5\times10^{-5}$	4.3×10^{-3} $\pm4.5\times10^{-5}$ *

Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group.

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Table 20: Effect of DMSO and NAC on liver and kidney weights in TCE-treated rats (Curative)

(Fig. 4.46, Fig. 4.47, Fig. 4.48 & Fig. 4.49: p. 194, p. 195 p. 196 & p. 197 respectively)

	Treatment 1					
Parameter	No treatment	Control	TCE 1.25ml/kg	DMSO 25%	DMSO 50%	DMSO 75%
liver weight (g/kg)	3.31×10^{-2} $\pm0.75\times10^{-3}$	3.30×10^{-2} $\pm0.71\times10^{-3}$	3.32×10^{-2} $\pm0.80\times10^{-3}$	3.22×10^{-2} $\pm1.58\times10^{-3}$	3.36×10^{-2} $\pm1.49\times10^{-3}$	3.44×10^{-2} $\pm0.99\times10^{-3}$
kidney weight (g/kg)	4.1×10^{-3} $\pm7.6\times10^{-5}$	4.3×10^{-3} $\pm5.7\times10^{-5}$	4.0×10^{-3} $\pm6.8\times10^{-5}$	4.0×10^{-3} $\pm11\times10^{-5}$	4.2×10^{-3} $\pm10\times10^{-5}$	4.1×10^{-3} $\pm13\times10^{-5}$

	Treatment 2					
Parameter	No treatment	Control	TCE 1.25ml/kg	NAC 150 mg/kg	NAC 300 mg/kg	NAC 600 mg/kg
liver weight (g/kg)	3.32×10^{-2} $\pm0.55\times10^{-3}$	3.31×10^{-2} $\pm0.73\times10^{-3}$	3.33×10^{-2} $\pm0.78\times10^{-3}$	3.40×10^{-2} $\pm0.36\times10^{-3}$	3.47×10^{-2} $\pm1.06\times10^{-3}$	3.38×10^{-2} $\pm0.54\times10^{-3}$
kidney weight (g/kg)	4.1×10^{-3} $\pm5.6\times10^{-5}$	4.3×10^{-3} $\pm6.3\times10^{-5}$	4.2×10^{-3} $\pm7.3\times10^{-5}$	4.1×10^{-3} $\pm9.5\times10^{-5}$	4.1×10^{-3} $\pm10\times10^{-5}$	4.0×10^{-3} $\pm12\times10^{-5}$

Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group.

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

4.5.3 The biochemical assays of the serum transaminase activity (Preventive).

Table 21: Effect of DMSO and NAC on CCl_4-induced elevation of SGPT and SGOT activities in rats (Preventive)

(Fig. 4.50, Fig. 4.51, Fig. 4.52 & Fig. 4.53: p. 198, p. 199 p. 200 & p. 201respectively)

			Treatment 1			
Parameter	No treatment	Control	CCl_4 1.25ml/kg	DMSO 25%	DMSO 50%	DMSO 75%
SGPT (IU/L)	12±0.5	11±0.7	1295 ±60.8	1295 ±58.8	1243 ±57.3	1079 ±81.1*
SGOT (IU/L)	52±1.0	53±1.9	1736 ±102	1726 ±45.9	1709 ±76.7	1591 ±61.6**

			Treatment 2			
Parameter	No treatment	Control	CCl_4 1.25ml/kg	NAC 150 mg/kg	NAC 300 mg/kg	NAC 600 mg/kg
SGPT (IU/L)	11±0.6	10±0.8	1283 ±77.0	1274 ±178	1296 ±69.5	1282 ±74.9
SGOT (IU/L)	52±1.0	52±1.3	1887 ±92.2	1775 ±138	1774 ±101	1596 ±129*

Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group.

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Table 22: Effect of DMSO and NAC on TCE-induced elevation of SGPT and SGOT activities in rats (Preventive)

(Fig. 4.54, Fig. 4.55, Fig. 4.56 & Fig. 4.57: p. 202, p. 203 p. 204 & p. 205 respectively)

			Treatment 1			
Parameter	No treatment	Control	TCE 1.25ml/kg	DMSO 25%	DMSO 50%	DMSO 75%
SGPT (IU/L)	13±0.7	12±0.5	15.89 ±1.07	14.88 ±0.91	15.00 ±0.91	15.01 ±0.82
SGOT (IU/L)	52±1.1	50±1.2	152.5 ±5.51	154.2 ±5.19	150.8 ±7.30	153.9 ±5.41

			Treatment 2			
Parameter	No treatment	Control	TCE 1.25ml/kg	NAC 150 mg/kg	NAC 300 mg/kg	NAC 600 mg/kg
SGPT (IU/L)	11±0.9	13±0.7	15.88 ±1.16	15.73 ±0.91	15.78 ±0.56	15.63 ±1.01
SGOT (IU/L)	53±0.5	51±1.0	150.0 ±6.52	152.2 ±5.39	153.9 ±7.34	147.0 ±7.48

Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group.

All values of SGPT and SGOT serum enzymes in all treated groups were comparable with ($p>0.05$) the values of the toxin control.

4.5.4 The organ weight. (Preventive)

Table 23: Effect of DMSO and NAC on CCl_4-induced increase of liver and kidney weights in rats (Curative)

(Fig. 4.58, Fig. 4.59, Fig. 4.60 & Fig. 4.61: p. 206, p. 207 p. 208 & p. 209 respectively)

	Treatment 1					
Parameter	No treatment	Control	CCl_4 1.25ml/kg	DMSO 25%	DMSO 50%	DMSO 75%
liver weight (g/kg)	3.38×10^{-2} $\pm0.74\times10^{-3}$	3.30×10^{-2} $\pm0.50\times10^{-3}$	5.08×10^{-2} $\pm0.94\times10^{-3}$	4.43×10^{-2} $\pm1.49\times10^{-3}$ **	4.39×10^{-2} $\pm1.13\times10^{-3}$ **	4.00×10^{-2} $\pm1.73\times10^{-3}$ **
kidney weight (g/kg)	4.1×10^{-3} $\pm4.6\times10^{-5}$	4.1×10^{-3} $\pm6.2\times10^{-5}$	4.4×10^{-3} $\pm7.9\times10^{-5}$	4.4×10^{-3} $\pm14\times10^{-5}$	4.4×10^{-3} $\pm8.3\times10^{-5}$	4.4×10^{-3} $\pm11\times10^{-5}$

	Treatment 2					
Parameter	No treatment	Control	CCl_4 1.25ml/kg	NAC 150 mg/kg	NAC 300 mg/kg	NAC 600 mg/kg
liver weight (g/kg)	3.32×10^{-2} $\pm0.66\times10^{-3}$	3.40×10^{-2} $\pm0.57\times10^{-3}$	5.22×10^{-2} $\pm0.78\times10^{-3}$	4.89×10^{-2} $\pm1.54\times10^{-3}$	4.29×10^{-2} $\pm1.20\times10^{-3}$ **	3.99×10^{-2} $\pm0.48\times10^{-3}$ **
kidney weight (g/kg)	4.0×10^{-3} $\pm5.5\times10^{-5}$	4.1×10^{-3} $\pm6.1\times10^{-5}$	4.5×10^{-3} $\pm9.7\times10^{-5}$	4.1×10^{-3} $\pm7.3\times10^{-5}$ *	4.1×10^{-3} $\pm7.8\times10^{-5}$ *	4.1×10^{-3} $\pm7.0\times10^{-5}$ *

Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group.

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Table 24: Effect of DMSO and NAC on liver and kidney weights in TCE-treated rats (Preventive)

(Fig. 4.62, Fig. 4.63, Fig. 4.64& Fig. 4.65: p. 210, p. 211 p. 212 & p. 213 respectively)

			Treatment 1			
Parameter	No treatment	Control	TCE 1.25ml/kg	DMSO 25%	DMSO 50%	DMSO 75%
liver weight (g/kg)	3.33×10^{-2} $\pm0.68\times10^{-3}$	3.34×10^{-2} $\pm0.77\times10^{-3}$	3.35×10^{-2} $\pm0.96\times10^{-3}$	3.36×10^{-2} $\pm1.35\times10^{-3}$	3.31×10^{-2} $\pm1.32\times10^{-3}$	3.44×10^{-2} $\pm0.76\times10^{-3}$
kidney weight (g/kg)	4.1×10^{-3} $\pm5.4\times10^{-5}$	4.2×10^{-3} $\pm7.4\times10^{-5}$	4.3×10^{-3} $\pm8.8\times10^{-5}$	4.3×10^{-3} $\pm16\times10^{-5}$	4.1×10^{-3} $\pm11\times10^{-5}$	4.3×10^{-3} $\pm12\times10^{-5}$

			Treatment 2			
Parameter	No treatment	Control	TCE 1.25ml/kg	NAC 150 mg/kg	NAC 300 mg/kg	NAC 600 mg/kg
liver weight (g/kg)	3.33×10^{-2} $\pm0.51\times10^{-3}$	3.33×10^{-2} $\pm0.57\times10^{-3}$	3.33×10^{-2} $\pm0.67\times10^{-3}$	3.44×10^{-2} $\pm0.92\times10^{-3}$	3.29×10^{-2} $\pm1.30\times10^{-3}$	3.48×10^{-2} $\pm1.04\times10^{-3}$
kidney weight (g/kg)	4.1×10^{-3} $\pm3.6\times10^{-5}$	4.2×10^{-3} $\pm5.3\times10^{-5}$	4.2×10^{-3} $\pm8.3\times10^{-5}$	4.1×10^{-3} $\pm18\times10^{-5}$	4.3×10^{-3} $\pm9.4\times10^{-5}$	4.3×10^{-3} $\pm5.1\times10^{-5}$

Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group.

All values of liver and kidney weights in all treated groups were comparable with ($p>0.05$) the values of the toxin control and saline-vehicle control.

4.6 Hepatoprotective effect of methanol extract of seaweeds against CCl_4- and TCE-induced poisoning in rats.

4.6.1 The biochemical assays of the serum transaminase activity (Curative).

Table 25: Effect of methanol extract of seaweeds on CCl_4 and TCE-induced elevation of SGPT and SGOT activities in rats (Curative)

(Fig. 4.66, Fig. 4.67, Fig. 4.68 & Fig. 4.69: p. 214, p. 215 p. 216 & p. 217 respectively)

					Treatment 1			
Parameter	No treatment	Control	CCl_4 1.25ml/kg	DMSO 25%	S#2 300mg/kg	S#3 300mg/kg	S#4 300mg/kg	---
SGPT (IU/L)	11 ±0.9	12 ±1.0	1235 ±116	1243 ±103	688.7 ±50.9*	1288 ±94.4	492.4 ±42.5**	---
SGOT (IU/L)	51 ±0.8	54 ±1.9	2119 ±89.0	2226 ±100	1630 ±186*	1682 ±69.2*	904.8 ±101**	---

					Treatment 2			
Parameter	No treatment	Control	TCE 1.25ml/kg	DMSO 25%	S#2 300mg/kg	S#3 300mg/kg	S#4 300mg/kg	Gal 300mg/kg
SGPT (IU/L)	11 ±0.8	12 ±0.8	15.78 ±1.03	14.87 ±1.16	14.04 ±0.82	10.52 ±0.32**	9.990 ±0.87**	11.52 ±0.67*
SGOT (IU/L)	53 ±0.3	51 ±1.7	153.0 ±6.50	140 ±2.03	89.41 ±3.53**	82.18 ±2.35**	111.7 ±5.55*	107.8 ±6.23**

Each value represents the mean±S.E.M. of 5 treated rats

* Significantly different when compared with the toxin control, $p< 0.05$.

** Significantly different when compared with the toxin control, $p< 0.005$.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

4.6.2 The organ weight. (Curative)

Table 26: Effect of methanol extract of seaweeds on liver and kidney weights in CCl_4 and TCE-treated rats

(Fig. 4.70, Fig. 4.71, Fig. 4.72 & Fig. 4.73: p. 218, p. 219 p. 220 & p. 221 respectively)

					Treatment 1			
Parameter	No treatment	Control	CCl_4 1.25ml/kg	DMSO 25%	S#2 300mg/kg	S#3 300mg/kg	S#4 300mg/kg	---
liver weight (g/lg)	3.33×10^{-2} $\pm0.48\times10^{-3}$	3.33×10^{-2} $\pm0.59\times10^{-3}$	5.23×10^{-2} $\pm1.11\times10^{-3}$	4.94×10^{-2} $\pm1.16\times10^{-3}$	4.94×10^{-2} $\pm1.06\times10^{-3}$	5.00×10^{-2} $\pm1.53\times10^{-3}$	5.01×10^{-2} $\pm1.06\times10^{-3}$	---
kidney weight (g/kg)	4.0×10^{-3} $\pm6.1\times10^{-5}$	4.1×10^{-3} $\pm7.1\times10^{-5}$	4.5×10^{-3} $\pm8.2\times10^{-5}$	4.4×10^{-3} $\pm8.5\times10^{-5}$	4.4×10^{-3} $\pm4.3\times10^{-5}$	4.6×10^{-3} $\pm7.8\times10^{-5}$	4.2×10^{-3} $\pm5.8\times10^{-5}$	---

					Treatment 2			
Parameter	No treatment	Control	TCE 1.25ml/kg	DMSO 25%	S#2 300mg/kg	S#3 300mg/kg	S#4 300mg/kg	Gal 300mg/kg
liver weight (g/lg)	3.30×10^{-2} $\pm0.70\times10^{-3}$	3.30×10^{-2} $\pm0.66\times10^{-3}$	3.34×10^{-2} $\pm0.88\times10^{-3}$	3.30×10^{-2} $\pm1.04\times10^{-3}$	3.22×10^{-2} $\pm1.02\times10^{-3}$	3.47×10^{-2} $\pm0.63\times10^{-3}$	3.26×10^{-2} $\pm1.18\times10^{-3}$	3.34×10^{-2} $\pm0.82\times10^{-3}$
kidney weight (g/kg)	4.2×10^{-3} $\pm5.4\times10^{-5}$	4.3×10^{-3} $\pm8.0\times10^{-5}$	4.2×10^{-3} $\pm6.5\times10^{-5}$	4.2×10^{-3} $\pm7.2\times10^{-5}$	4.2×10^{-3} $\pm9.0\times10^{-5}$	4.2×10^{-3} $\pm2.7\times10^{-5}$	4.1×10^{-3} $\pm14\times10^{-5}$	4.2×10^{-3} $\pm10\times10^{-5}$

Each value represents the mean±S.E.M. of 5 treated rats

All values of liver and kidney weights in: 1. CCl_4-treated groups were comparable with ($p>0.05$) the values of the toxin control; 2. TCE-treated groups were comparable with ($p>0.05$) the values of the toxin control and saline-vehicle control.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

4.7 Histopathological examinations.

In order to quantify the results, a grading system was adopted (Portmann et al., 1975). This grading system is based on the histological change of the liver, especially the extent of the damaged area around the central vein. The scheme is shown as follows:

Main histological features	**Grade**
Normal, no change	0
Swelling of liver cell nuclei, minor vacuolation, no necrosis.	+
Single cell necrosis in centrilobular hepatocytes	++
Confluent necrosis of centrilobular hepatocytes	+++
Confluent necrosis in continuity between adjacent lobules	++++
Massive necrosis leaving rim of liver cells around portal tracts	+++++

4.7.1 Acute hepatotoxicity test on aqueous seaweed extracts.

Light microscopy

All sections revealed no special difference when compared with the vehicle-saline control or no treatment groups in the curative test (0).

a. No treatment group (Fig. 4.74 to Fig. 4.76: p. 222 to p. 224)

No any treatments were given, food and water fully accessible except food which was withdrawn approximately 18 h before experiment. No sign of abnormalities were noted (0).

Well distributed of hepatocyte cords around the central vein with the clear shown of sinusoids were noted. The healthy liver cells with clear stained of nucleus and cytoplasm were also observed.

b. Vehicle-saline group control, curative (Fig. 4.77 to Fig. 4.79: p.224 to p. 226)

vehicle, corn oil and saline (0.9% v/v) were fed to the animals. No sign of abnormalities were noted (0).

Well distributed of hepatocyte cords around the central vein with the clear shown of sinusoids and sinusoidal cells were noted. The healthy liver cells with clear stained of nucleus and cytoplasm were also observed (no difference when compared with the no treatment group).

4.7.2 Curative and preventive tests of seaweed aqueous extracts against the CCl_4-induced hepatotoxicity.

Light microxcopy

a. No treatment group (Refer to section 4.7.1)

b. Vehicle-saline group control, curative (Refer to section 4.7.1)

c. Toxin control, curative (Fig. 4.80 to Fig. 4.82: p. 228 to p. 230)

CCl_4 (1.25 ml/kg) and saline (0.9% v/v) were fed to the animals. Serious necrosis was noted (+++++).

The liver showed hydropic swelling and massive necrosis of hepatocytes in the centrilobular area (cv) with fat accumulation. Massive centrilobular necrosis was extended to the portal tract, leaving a rim of cells appeared normal only. Swollen cells were identified by enlargement and the pale staining of cytoplasm (balloon cells) and necrotic cells were identified by pyknotic nuclei and ruptured plasma membrane. Cells containing lipid were identified by round droplets within the cytoplasm in high power micrograph (x293). Liver vacuolization and serious sinusoidal congestion with the condensed nucleus were evidenced. Well distributed of hepatocyte cords around the central vein cannot be seen.

d. CCl_4 + aqueous extract of *S. henslowianum*, curative. (Fig. 4.83 to Fig. 4.88: p. 230 to p. 236)

***S. henslowianum* (15 mg/ml saline)**

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of aqueous seaweed extract orally, *S. henslowianum* (15 mg/ml saline). Serious necrosis was noted (+++++).

The liver showed hydropic swelling and massive necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was extended to the portal tract, leaving a rim of cells appeared normal only. Moreover, necrotic area was observed near the central vein. Swollen cells, pyknotic nuclei and ruptured plasma membrane were identified. Liver vacuolization and serious sinusoidal congestion with the condensed nucleus were evidenced in high power micrograph (x194). Well distributed of hepatocyte cords around the central vein cannot be seen. As a result, the *S. henslowianum* (15 mg/ml saline) extract exhibited the effect similar to the toxin control.

***S. henslowianum* (60 mg/ml saline)**

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of aqueous seaweed extract orally, *S. henslowianum* (60 mg/ml saline). mild necrosis was noted (++).

The liver showed hydropic swelling and a very little necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was not noted. Moreover, a clear shown of necrotic area was not noted near to the central vein. Few amounts of swollen cells could be observed. In addition, a large range of regeneration zone around the central vein accompanied by the present of mitotic figures. Liver vacuolization and sinusoidal congestion were still evidenced in high power micrograph (x194). But, the condition was not as severe as toxin control. Well distributed of hepatocyte cords around the central vein could be seen close to the central vein. As a result, the *S. henslowianum* (60 mg/ml saline) extract exhibited

excellent hepatoprotective effect to relieve the CCl_4-induce hrepatotoxicity.

e. CCl_4 + aqueous extract of *Myagropsis myagroides*, curative. (Fig. 4.89 to Fig. 4.93: p.236 to p. 240)

***Myagropsis myagroides* (15 mg/ml saline)**

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of aqueous seaweed extract orally, *Myagropsis myagroides* (15 mg/ml saline). Serious necrosis was noted (+++++).

The liver showed hydropic swelling and massive necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was extended to the portal tract, leaving a rim of cells appeared normal only. Moreover, necrotic area was observed near to the central vein. Swollen cells, pyknotic nuclei and ruptured plasma membrane were identified. Liver vacuolization and serious sinusoidal congestion with the condensed nucleus were evidenced in high power micrograph (x297). Moreover, well distributed of hepatocyte cords around the central vein cannot be seen. As a result, the *Myagropsis myagroides* (15 mg/ml saline) extract exhibited the effect similar to the toxin control and *S. henslowianum* (15 mg/ml saline) extract.

In the *Myagropsis myagroides* (15 mg/ml saline) extract-treated rat's liver section selected. The evidence of regeneration zone appeared to increasing as compared with the 15 mg/ml saline-treated one.

***Myagropsis myagroides* (60 mg/ml saline)**

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of aqueous seaweed extract orally, *Myagropsis myagroides* (60 mg/ml saline). mild necrosis was

noted (between ++ and +++).

The liver showed hydropic swelling and a few necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was not being noted. Moreover, a clear shown of necrotic area was being noted near to the central vein. However, the condition was less severe than 15 mg/ml saline one. A number of swollen cells could be observed. In addition, a large range of regeneration zone around the central vein accompanied by the present of mitotic figures. Liver vacuolization and sinusoidal congestion were still evidenced in high power micrograph ($\times$194). But, the condition was not as severe as toxin control. Well distributed of hepatocyte cords around the central vein could not be seen close to the central vein. As a result, the *Myagropsis myagroides* (60 mg/ml saline) extract exhibited a good hepatoprotective effect to relieve the CCl_4-induce hrepatotoxicity. And it was not as good as *S. henslowianum* (60 mg/ml saline) extract.

f. CCl_4 + aqueous extract of *S. siliquastrum*, curative. (Fig. 4.94 to Fig. 4.97: p. 242 to p. 244)

***S. siliquastrum* (15 mg/ml saline)**

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of aqueous seaweed extract orally, *S. siliquastrum* (15 mg/ml saline). Serious necrosis was noted (+++++).

The liver showed hydropic swelling and massive necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was extended to the portal tract, leaving a rim of cells appeared normal only. Moreover, necrotic area was observed near to the central vein. Swollen cells, pyknotic nuclei

and ruptured plasma membrane were identified. Liver vacuolization and serious sinusoidal congestion with the condensed nucleus were evidenced in high power micrograph (x297). Moreover, well distributed of hepatocyte cords around the central vein cannot be seen. As a result, the *S. siliquastrum* (15 mg/ml saline) extract exhibited the effect similar to the toxin control, *S. henslowianum* and *Myagropsis myagroides* (15 mg/ml saline) extracts.

S. siliquastrum (60 mg/ml saline)

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of aqueous seaweed extract orally, *S. siliquastrum* (60 mg/ml saline). Necrosis was noted (++++).

The liver showed hydropic swelling and a moderate necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was not being noted. Moreover, a clear shown of necrotic area was being noted near to the central vein. However, the condition was less severe than 15 mg/ml saline one. A number of swollen cells could be observed. In addition, a large range of regeneration zone around the central vein accompanied by the present of mitotic figures.. But, the condition was not as good as the same dosages of the other two seaweed extracts. Well distributed of hepatocyte cords around the central vein could not be seen close to the central vein. As a result, the *S. siliquastrum* (60 mg/ml saline) extract exhibited fair hepatoprotective effect to relieve the CCl_4-induce hepatotoxicity. And it was not as good as *S. henslowianum* and *Myagropsis myagroides* (60 mg/ml saline) extract.

Light microscopy of H & E-stained kidney sections taken at 24 h after CCl_4 administration to toxin control rats, vehicle-saline control and all other treatments

rats whatever curative or preventive, did not reveal any significant morphological alterations **(Fig. 4.98 & Fig. 4.99: p. 246)**. All treatment shown revealed no difference when compared with the vehicle-saline control.

g. Toxin control, preventive. (Fig. 4.100 & Fig. 4.101: p. 248)

CCl_4 (1.25 ml/kg) and saline (0.9% v/v) were fed to the animals. Serious necrosis was noted (+++++). The result is similar to the toxin control in curative one.

h. CCl_4 + aqueous extracts, preventive. (Fig. 4.102 & Fig. 4.103: p. 250)

CCl_4 (1.25 ml/kg) and saline (0.9% v/v) were fed to the animals before the 10 ml/kg of aqueous seaweed extract orally. Serious necrosis was noted (+++++) in all sections.

All aqueous seaweed extracts treatment exhibited the effect similar to the toxin control in preventive test. Even the most effective of *S. henslowianum* extract (60 mg/ml saline) in curative test still revealed the extensive necrosis of hepatocytes around the central vein region in the preventive test. And the *S. siliquastrum* extract (60 mg/ml saline) exhibited the similar effect to the *S. henslowianum* extract (60 mg/ml saline) one. So, all treatment shown revealed no signs of hepatoprotective effect.

SEM microscopy **(Fig. 4.104 to Fig. 4.107: p. 252 to p. 254)**

a. Vehicle-saline group control, curative.

Vehicle, corn oil and saline (0.9% v/v) were fed to the animals. No signs of abnormalities were noted.

Normal hepatocytes cords linings with clear shown of normal nuclei and

sinusoids could be seen.

b. Toxin control, curative.

CCl_4 (1.25 ml/kg) and saline (0.9% v/v) were fed to the animals. Serious necrosis was noted with clear shown of necrotic cell and group of vacuolization close around the central vein. That damage features could be seen clearly in high power micrographs **(Fig. 4.106 & Fig. 4.107: p. 254)**.

TEM microscopy **(Fig. 4.108 to Fig. 4.111: p. 256 to p. 258)**

a. Vehicle-saline group control, curative.

vehicle, corn oil and saline (0.9% v/v) were fed to the animals. No signs of abnormalities were noted.

TEM observations of vehicle-saline control revealed no sign of alteration in the ultrastructure of the hepatocytes. Clear shown of mitochondria and rough endoplasmic reticulum (rER) etc. expressed the sign of healthy stage.

b. Toxin control, curative (Fig. 4.112 to Fig. 4.116: p. 260 to p. 264)

CCl_4 (1.25 ml/kg) and saline (0.9% v/v) were fed to the animals. Serious necrosis was noted.

TEM observations of toxin control revealed serious sign of alteration in the ultrastructure of the hepatocytes. The increase of intracellular lipid and the swollen of mitochondria and rough endoplasmic reticulum could be seen clear in all magnifications. The early and final stages of injury could be seen clearly by the reveal of the swollen of mitochondria and necrotic cell respectively. In the highest magnification, the swollen mitochondria, with roughly granular appearance and lipid droplet; the dilation and fragmentation of the rER, could be seen.

4.7.3 Acute hepatotoxicity test of TCE in rats by oral and intraperitoneal routes

(Fig. 4.117 to Fig. 4.125: p. 266 to p. 274)

All liver sections exhibited no abnormality when compared with the vehicle-saline control. Even the effective dose of TCE (20% TCE, 1.25 ml/kg) also revealed no special difference when compared with the vehicle-saline control (0).

Light microscopy of H & E-stained kidney sections taken at 24 h after 20% TCE effective administration to toxin control rats, did not reveal any significant morphological alterations in oral or i.p. routes except 30% TCE in i.p. route one, showing abnormal kidney cells. Actually, there was no necrosis in the renal cortex. Moreover, no reveal of morphological change in glomerulus. However, distal and proximal tubules dilation together with the cell debris could be seen clearly in the low and medium power micrographs **(Fig. 4.120 & Fig. 4.121: p. 270)**. In addition, cell debris and detachment of brush border could also be observed in the proximal tubules from the high power micrographs **(Fig. 4.122 & Fig. 4.123: p. 272)**.

SEM microscopy **(Fig. 4.124 & Fig. 4.125: p. 274)**

In the 20% TCE effective dose treatment. No reveal of abnormality could be seen in the liver SEM liver sections. Clear shown of hepatocytes, sinusoids and normal nucleus could be seen. There was no difference when compared with the vehicle-saline control in curative test.

TEM microscopy **(Fig. 126 & Fig. 129: p. 276 & p. 278)**

TEM observations of 20% TCE effective dose revealed no special sign of alteration in the ultrastructure of the hepatocytes. Clear shown of mitochondria and rER and other organelles could be seen clearly. However, the increase number of

vacuoles were observed in certain areas **(Fig. 4.127: p. 276)**. Moreover, in the highest magnification, no sign of abnormality was seen in the mitochondria. However, swollen rER could be seen clearly.

4.7.4 Curative and preventive tests of seaweed aqueous extracts against the TCE effective dose-induced toxicity.

Based on the result of light microscopy from section 4.7.3. aqueous seaweed extracts treatment at the highest dosage (60 mg/ml saline) were selected to study in light microcopy (liver and kidney sections). No reveal of abnormality could be seen. And the result drawn from the study is no special difference when compared with the vehicle-saline control group (Liver sections: (0)).

4.7.5 Antidotal effects of dimethyl sulfoxide (DMSO) and N-acetylcysteine (NAC) against CCl_4- and TCE-induced poisoning in rats.

Light microscopy

a. CCl_4 + DMSO, curative. (Fig. 4.130 to Fig. 4.132: p. 280 to p. 282)

DMSO 25% in saline

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of DMSO 25% orally. Serious necrosis was noted (+++++).

The liver shown exhibited the morphological changes similar to the toxin control. The massive necrosis closed to the central vein, necrotic cells, swollen cells and vacuolization could all be seen close around the central vein.

DMSO 75% in saline

CCl_4 (1.25 ml/kg) was fed to the animals, followed by DMSO 75% orally, moderate necrosis was noted (+++).

The liver showed hydropic swelling and a moderate necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was not being noted. However, a clear shown of necrotic area was noted near to the central vein. Few amount of swollen cells could be observed. In addition, a large range of regeneration zone around the central vein accompanied by the present of mitotic figures. Liver vacuolization and sinusoidal congestion were still evidenced in high power micrograph (x194). But, the condition was not as severe as toxin control. Well distributed of hepatocyte cords around the central vein could be seen close to the central vein. As a result, the DMSO 75% dosage exhibited a good hepatoprotective effect to relieve the CCl_4-induce hrepatotoxicity.

b. CCl_4 + NAC, curative. (Fig. 4.133 to Fig. 4.139: p. 282 to p. 288)

NAC (15 mg/ml saline)

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of NAC (15 mg/ml saline) orally. Serious necrosis was noted (+++++).

The liver shown exhibited the morphological changes similar to the toxin control. The massive necrosis closed to the central vein, necrotic cells, swollen cells and vacuolization could all be seen close around the central vein.

In NAC (30 mg/ml saline) treatment, the sign of regeneration can be seen as the result of increase the area of regeneration zone **(Fig. 4.136: p. 286)**.

NAC (60 mg/ml saline)

CCl_4 (1.25 ml/kg) was fed to the animals, followed by NAC (60 mg/ml saline) orally, moderate necrosis was noted (++).

The liver showed hydropic swelling and a mild necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was not being noted. Although a clear shown of necrotic area was noted, it was only in a mild condition. Few amount of swollen cells could be observed. In addition, a large range of regeneration zone around the central vein accompanied by the present of mitotic figures. Liver vacuolization and sinusoidal congestion were still evidenced in high power micrograph (x297). But, the condition was not as severe as toxin control. As a result, the NAC (15 mg/ml saline) dosage exhibited excellent hepatoprotective effect to relieve the CCl_4-induce hrepatotoxicity.

The DMSO and NAC treatment in CCl_4, preventive test, showed massive necrosis around the central vein (+++++). The results were similar to the toxin control group (curative or preventive). Besides, no morphological change could be seen in the renal cortex of kidney sections.

The DMSO and NAC treatment in TCE, curative or preventive, all showed no morphological difference in liver and kidney sections when compared with the vehicle-saline control (Liver sections: (0)).

4.7.6 Hepatoprotective effect of methanol extract of seaweeds against CCl_4- and TCE-induced poisoning in rats.

a. Toxin control, curative

CCl_4 (1.25 ml/kg) and saline (0.9% v/v) were fed to the animals. Serious necrosis was noted (+++++).

The result of morphological changes in this toxin group was similar to the toxin control group (**Fig. 4.80 to Fig. 4.82: p. 228 to p. 230**).

b. CCl_4 + methanol extract of *S. henslowianum*, curative (Fig. 4.140 & Fig. 4.141: p. 290)

***S. henslowianum* (30 mg/ml 25% DMSO)**

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of aqueous seaweed extract orally, *S. henslowianum* (30 mg/ml 25% DMSO). Moderate necrosis was noted (+++).

The liver showed hydropic swelling and moderate necrosis of hepatocytes in the centrilobular area. Massive centrilobular necrosis was not noted. Necrotic area was observed quite far away to the central vein. Swollen cells and ruptured plasma membrane were identified. Slightly liver vacuolization and was evidenced in high power micrograph (x194). Although well distributed of hepatocyte cords around the central vein cannot be seen, a large range of regeneration zone around the central vein accompanied by the present of mitotic figures. As a result, the *S. henslowianum* extract (15 mg/ml 25% DMSO) exhibited certain effect to act against the CCl_4-induced hepatotoxicity.

***Myagropsis myagroides* (30 mg/ml 25% DMSO) (Fig. 4.142 & Fig. 4.143: p. 292)**

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of methanol seaweed extract orally, *M. myagroides* (30 mg/ml 25% DMSO). Serious necrosis

was noted (between +++ and ++++).

The liver showed hydropic swelling and massive necrosis of hepatocytes in the centrilobular area with fat accumulation. Moreover, necrotic area was observed near to the central vein. Swollen cells and ruptured plasma membrane were identified. Liver vacuolization and serious sinusoidal congestion were evidenced in high power micrograph (x 188). Moreover, well distributed of hepatocyte cords around the central vein cannot be seen. The necrotic cells were close to the central vein. However, a mitotic figure was identified to indicate the liver cells may have certain degree or regeneration. As a result, the *Myagropsis myagroides* (30 mg/ml 25% DMSO) extract exhibited the least or no hepatoprotective effect to the CCl_4-induced hepatotoxicity.

***S. siliquastrum* (30 mg/ml 25% DMSO) (Fig. 4.144 & Fig. 4.145: p. 294)**

CCl_4 (1.25 ml/kg) was fed to the animals, followed by 10 ml/kg of aqueous seaweed extract orally, *S. siliquastrum* (30 mg/ml 25% DMSO). Mild necrosis was noted (between ++ and +++).

The liver showed hydropic swelling and a few necrosis of hepatocytes in the centrilobular area with fat accumulation. Massive centrilobular necrosis was not being noted. Moreover, a clear shown of necrotic area was not being noted. Few amount of swollen cells could be observed. In addition, a large range of regeneration zone around the central vein accompanied by the present of mitotic figures could be observed. Liver vacuolization and sinusoidal congestion were still evidenced in high power micrograph (x 192). But, the condition was not as severe as toxin control. Although well distributed of hepatocyte cords around the central vein could not be seen close to the central vein, the potential of recovery could be seen by the increase

number of mitotic figures mentioned above. As a result, the *S. siliquastrum* (30 mg/ml 25% DMSO) extract exhibited very good hepatoprotective effect to relieve the CCl_4-induce hepatotoxicity as compared with the other two methanol extracts of seaweed.

Chapter 5 DISCUSSION

Acute hepatotoxicity test on aqueous seaweed extracts

Determination of serum activities of hepatic enzymes released from the injured liver has become one of the useful experimental tools in evaluation of hepatotoxicity (Plaa & Hewitt, 1982). Moreover, the increase in the activities of SGPT and SGOT is considered as a reliable index of liver damage (Wang et al., 1996). In the present study, the preliminary tests of the possible hepatotoxicity of aqueous extract seaweeds were done. The result from biochemical analyses and histopathological examinations showed that all aqueous seaweed extracts in different dosages would not cause any biochemical and histopathological change in both liver and kidney of the rats. As a result, all aqueous extracts used in the present study by oral administration may be assumed to have no hepatotoxic or nephrotoxic effects in rats at the range of dosages used.

Curative and preventive tests of seaweed aqueous extracts against the CCl_4-induced hepatotoxicity.

CCl_4 is commonly used as a toxin model to evaluate hepatotoxicity (Plaa & Hewitt, 1982). When the liver is injured as a result of the introduction of infectious agents or chemicals, the serum levels of SGPT and SGOT are raised significantly, accompanied with the increase in weight of both liver and kidney (Kluwe, 1981; Iglesia et al., 1982). The increase in SGPT and SGOT levels have been attributed to damage to the structural integrity of the liver (Chenoweth & Hake, 1962). They may

be released from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage (Sallie et al., 1991). At a suitable dosage, CCl_4 causes extensive necrosis in the liver centrilobular regions around the central veins (Slater, 1966). The mechanism of CCl_4 hepatotoxicity is considered to be the result from activation of CCl_4 by cytochrome P-450 system in the hepatocyte's endoplasmic reticulum to the reactive metabolite, $CCl_3^{\cdot}$, which can form covalent products with macromolecules and lipid and interact with O_2 to generate $CCl_3O_2^{\cdot}$ which in turn initiates lipid peroxidation of membrane lipids and the endoplasmic reticulum rich in polyunsaturated fatty acids (Slater, 1966; Recknagel, 1967; Klaassen & Plaa, 1969; Packer et al., 1978; Gilani & Janbaz, 1995a, b and c; Jazbaz & Gilani, 1995;), causing the disintegration of lysosomal, mitochondrial and cellular membranes and leading finally to cell necrosis (Slater, 1972; Recknagel et al., 1982). After administration of a single dosage of CCl_4 given by gavage, the centrilobular necrosis begins to develop with evidence of the lesion by 12 h and full-blown necrosis by 24 h. The liver may be restored to normal within 14 days with the removal of the residues of necrotic tissue (Smuckler, 1975). As a result, the 24 hours period is well adopted as a toxicity end-point.

In the present investigation, there are considerable increases in the serum levels of SGPT and SGOT in toxin control rats, which are significantly different from those of both the vehicle-saline control group and no treatment group. This in turn indicated that there was damage of the organ to which the enzymes are specific. Moreover, the liver and kidney weights also increase significantly after the application suitable dosage (1.25 ml/kg) of 20% CCl_4 applied.

The hepatotoxicity in biochemical analysis could be compared with that of the histopathological studies. Histopathological studies further provide detailed information about the degree of hepatic lesion. In the light microscopy observations, acute liver damage induced by CCl_4 in the toxin control group can be illustrated by the presence of massive centrilobular necrosis around the central veins, ballooning degeneration and cellular infiltration as well as fat accumulation of the liver. The massive centrilobular necrosis is extended to the portal tract, leaving a rim of cells appeared normal only. These results can be seen in both curative and preventative tests. Thus, the CCl_4 applied could cause the acute injury of liver biochemically and histologically. In SEM observations, the three dimensional structure of rat liver can be seen (Gaudio et al., 1993). The presence of necrotic cells and groups of vacuolization were clearly seen on the liver fracture. The normal nucleus and well distributed of hepatocyte cords with clear pattern of sinusoids cannot be noted when compared with SEM samples of the vehicle-saline control group. In TEM observations, it can yield clues to the mechanisms of hepatotoxicity by studying the structural changes and rate of its development induced by toxic agents (Zimmerman, 1982). The TEM micrographs shown could illustrate the rate of CCl_4 toxicity development (**Figs. 4.112 to 4.116: p. 260 to p. 264**). The increase of intracellular lipids; mitochondrial swelling, dilation; swelling and fragmentation of rough endoplasmic reticulum as well as the necrosis of the whole cell unit could be elicited by CCl_4-induced hepatotoxicity. As reported previously, CCl_4 can induce fatty liver associated with fat accumulation (Reynolds, 1963). The probable sites of extraneous free radical formation are the mitochondria and the endoplasmic reticulum initially (Slater, 1966). However, mitochondrial swelling has been described in the early stage of CCl_4 intoxication (Recknagel & Malamed, 1956), though a structure

bounded by a single lipoprotein membrane (endoplasmic reticulum) would be far more sensitive to damage induced by free radical attack than a double-layered membrane as in the mitochondria (Slater, 1966). In addition, changes of mitochondria can reveal the rate of mechanism of CCl_4 toxicity by its effect on (1) mitochondrial membrane, which either disrupts or else simply fuses to become single, though unbroken; (2) the cristae, which break up into granulation and disappear; (3) the matrix, which assumes a roughly granular or filamentous appearance and the final phase of mitochondrial transformations into lipid droplets in hepatic cell (Rouiller, 1964). The liver sections of vehicle-saline control group showed a normal cell pattern of hepatocyte cords with well-shown of sinusoids and normal nucleus in SEM. Moreover, the clear fine structure of normal organelles inside the cells can also be revealed by TEM. The biochemical and histopathological studies aim to successfully interpret the mechanisms culminating in necrosis (Slater, 1966). As a result, the detection of early damage from measurement of enzyme levels of serum in the biochemical tests can fully complement with the histopathological examinations in light microscopy, SEM and TEM, which show the acute elevated levels of SGPT and SGOT accompanied with a severe centrilobular necrosis and fatty degeneration of liver after the administration of a suitable dosage (1.25 ml/kg) of 20% CCl_4 to rats (Slater, 1966). The present results in the acute CCl_4-induced hepatic injuries could be used as a model for the screening of hepatoprotective agents (Plaa & Hewitt, 1982).

Of the three species of seaweeds under study, the aqueous extracts of *S. henslowianum* and *M. myagroides* administered at all three dosages (150 mg/kg, 300 mg/kg and 600 mg/kg saline) exhibited significant hepatoprotection against CCl_4-induced liver injury in rats by reducing the acute increase of SGPT and SGOT

levels. *S. siliquastrum* also showed significantly hepatoprotective effect but only at the higher dosages. Of these three seaweed species, *S. henslowianum* appeared to be the most effective and promising in the histopathological examination to show the least necrosis when compared with *S. siliquastrum*. However, little necrotic cells could still be found far away from the central vein. The similar curative effects of that extracts could also be seen to treat against the CCl_4-induced enlargement of the liver weights. Nevertheless, the significantly reduced of kidney weight could be seen only when the highest dosage of *S. henslowianum* extract was applied. According to the histopathological study of the kidney sections, although there was a significant increase of kidney weight in the toxin control group, histopathological changes in the sections were not detected. It is probably due to the reason that many common strains of laboratory rats are relatively resistant to acute nephrotoxic effects of CCl_4 and the reason is still unclear (Striker et al., 1968; Kluwe, 1982; Elfarra, 1993). Therefore, the liver could well be illustrated to be the main target site of CCl_4-induced toxicity in this test.

Based on the preventive test of the aqueous extracts on CCl_4 hepatotoxicity, there was significant decrease in the serum levels of SGPT in rats treated with *M. myagroides* extracts in three different dosages. However, no significant difference can be seen in their SGOT levels. It indicated that *M. myagroides* offered a significant hepatoprotection to prevent the acute elevation of SGPT. As SGPT is specific in the liver, the aqueous extracts of *M. myagroides* may exhibit a good effect in preventive purpose. The same result can also be seen in the effects to reduce the acute increase in weights of liver and kidney. Nevertheless, the massive necrosis could still be found in the histopathological examination of the liver in all treatments

even in the most effective one, *S. henslowianum* (60 mg/ml saline). Therefore, preventive effects of seaweed extracts were not good enough to protect the liver against the acute CCl_4-induced hepatotoxicity. Assumably, the aqueous extracts of seaweeds in the preventive test may not exhibit the protective effect to preserve the structural integrity of the hepatocellular membrane against CCl_4. Besides, the result of SGPT levels and liver weight analysis of *S. siliquastrum* indicated it had the least effect in preventing the acute CCl_4-induced hepatotoxicity. Based on the result of SGPT level at dosage of 600 mg/kg saline, it probably exerted a certain degree of adverse effect to the liver in this stage, as a significant difference was shown in SGPT level which was higher than that of the toxin control. Owing to the fact that SGPT is more specific in the liver, the increase of this enzyme levels may attribute to the damage of liver cells. Furthermore, the effect of *S. siliquastrum* extract exhibited only a little or no hepatoprotctive effect in the enzyme assays and histopathological examination throughout the curative and preventive test against CCl_4-induced hepatotoxicity. Therefore, it may be regarded as a negative control. The significant raise in SGPT in the preventative test may be attributed to some experimental adverse condition.

The hepatoprotective effects of seaweed in preventive mode was not as good as those in the curative mode. The main possible reason may be due to the aqueous nature of the extract of seaweeds. They may be absorbed and excreted easily from the rat. Besides, the dosage applied to the rats in preventive mode was given 6 hours before the toxin treatment. Most or all of the potential active compound(s) may be excreted out from the body through urination or other biochemical mechanisms. The residues inside the body may not exert a significant protective effect to relieve the

CCl_4-induced heaptotoxicity. Some preventive studies performed require the experimental animals to receive several doses of potential antidote for about 3 consecutive days before toxin treatment (Montilla et al., 1990; Gilani & Janbaz, 1995b).

The aqueous seaweed extracts (*M. myagroides, S. henslowianum and S. siliquastrum*) probably acted to preserve the structural integrity of the plasma cellular membrane of the hepatocytes to protect it from breakage by the reactive metabolites produced. *S. henslowianum* appears to have the best overall curative action, followed by *M. myagroides* and *S. siliquastrum.* Furthermore, the hepatoprotective activity of the extracts is also possibly due to their antioxidant properties; acting as scavengers of free radicals such as superoxide and alkoxy radicals (Ooi, 1996). The curative action on hepatic injury by the crude seaweed extracts may have been due to the presence of some active components which can protect the liver against liver plasma membrane alteration or promote cellular mitosis action for the repair of the liver cells. Some of these may involve active binding sites as indicated by the saturation of their effects with higher concentration of seaweed extracts.

TCE toxicity test

In the TCE toxicity test, TCE administered in a single or twice oral dosage (40% of TCE) exhibited the significant effects in all tested parameters. Although the biochemical analysis and organ weights of both liver and kidney also showed that there were abnormalities when given certain percentages of TCE, no histopathological changes in both liver and kidney sections at those dosages or even

in other dosages were observed. Therefore, it indicated that the main target site of TCE might not be the liver or kidney at those dosages. Threshold limit of mortality is around 40% TCE or above.

Based on the TCE toxicity test by i.p. route, the induction of toxicity became more prominent. From 20% TCE onwards, the significant elevated levels of SGPT and SGOT were clearly shown. The values of enzyme levels increased if the percentage of TCE increased. Dose-response relationship could roughly be achieved. However, this relationship would not be obtained from 30% to 40% TCE administration as the SGPT and SGOT elevation appeared to have a trend to level off. It might be due to the approach of the maximum tolerance point (threshold) around the 40% of TCE. Treated animals died when the threshold was reached or beyond. The significant change in kidney weight was noted when 40% of TCE was applied. Besides, the presence of significant histopathological changes in kidney cortex could also be detected from the 30% TCE onwards. The renal injuries consisted of the degeneration and dilation of the convoluted tubules (proximal and distal tubules) with the plugging of cellular debris in the lumen. However, conspicuous difference in the liver structure or liver weight was observed in all toxin treatments when compared with the vehicle-saline control group. As a result, the liver might not be the main target site for the TCE toxicity in i.p. route. Based on the analysis of SGPT and SGOT levels, the elevation of these enzymes indicated that the toxicity was generated from the liver, especially the levels of SGPT which is specific in the liver. However, no significant effect was found as analysed on histopathological data and liver weight. In addition, although there was no significant change in kidney weight after these TCE treatment, significant toxicological effect in the kidney proximal and

distal tubules accompanied with the decrease in the kidney weight were found in 30% TCE treatment group. It indicated that the kidney may be the target sites prior to the liver at this dosage. This is in agreement with some research that has indicated little or no hepatotoxic effects following TCE exposure (Waters, 1977). Moreover, it was reported that the induction of liver lesion and transaminase activities could only be achieved after the pretreatment of phenobarbital (Allemand et al., 1978; Rouisse & Chakrabarti, 1996). According to the result of Allemand et al. (1978), TCE itself does not bind to proteins. However, phenobarbital-pretreated rats causes the covalent binding of a chemically reactive metabolite of TCE to hepatic proteins of the liver tissue where it is formed by a cytochrome P-450-dependent reaction. The cytochrome P-450 isozymes such as CYP2E1, involve effectively in the TCE biotransformation (Hanioka et al., 1997). As a result, cell necrosis may be due to the formation of reactive metabolite(s). Hepatic glutathione decreases after TCE administration to the non-pretreated rats, and it has been suggested that TCE may be metabolized into a glutathione conjugate (Reynold et al., 1975). Based on *in vitro* test, the addition of glutathione to the incubation mixture decreases the amount of TCE metabolite bound to microsomal proteins (Allemand et al., 1978). It was that proposed a chemically reactive metabolite(s) formed by cytochrome P-450, trichloroethylene epoxide, which reacts with and binds to either proteins or glutathione to cause the toxicity.

In the present TCE toxicity study by oral and i.p. route, it may be further concluded that the reactive metabolite formed: 1) may not be adequate enough to cause the necrosis by binding to proteins, or 2) may prefer binding with glutathione. As one type of nephrotoxic glutathione S-conjugates is exemplified by the conjugates

of TCE (Elfarra, 1993). The nephrotoxicity seen in rats appears to attribute to a second minor pathway involving glutathione conjugation of TCE (Green et al., 1997). The S-(1,2-dichlorovinyl)-L-cysteine (DCVC) and S-(1,2-dichlorovinyl)glutathione (DCVG) formed *in vivo* from that pathway are extremely low in rats and appears relatively insignificant when compared to the concentration of S-conjugates formed *in vivo* by other nephrotoxic haloalkenes such as dichloroacetylene (Dekant et al., 1990; Green et al., 1997). As a result, the metabolic formation of DCVC and DCVG from TCE in rats may readily explain the acute nephrotoxicity observed after high dosages of TCE applied (Dekant et al., 1990). The nephrotoxicity of these conjugates requires cleavage of the corresponding cysteine conjugates by cysteine conjugate β-lyase to generate reactive thiols which are believed to be the proximate or ultimate toxic species (Elfarra, 1984). Therefore, the nephrotoxicity of TCE found in certain dosages by i.p. route may attribute to the conjugation of TCE. Owing to the reason of minor pathway and extremely low concentration of conjugates formed. The significant nephrotoxicity generated could be observed only in higher dosages by i.p. (1.25 ml/kg of 30% TCE or higher). According to some related experiments, TCE may cause death of rats if a threshold is reached. Beyond this threshold injury progresses, culminating in organ failure and animal death as the final stage (Mangipudy et al., 1995; Rao et al., 1997; Soni et al., 1998).

In addition, the effective dose of TCE by i.p. route which could generate significant elevation of SGPT and SGOT with no mortality of tested animals, was more prominent than via oral administraion. It was probably due to the toxic agents administered by i.p. route generally elicited greater effect and produced more rapid response than by oral route (Klaassen & Eaton, 1991). Therefore, it may also be used as a toxin model for the evaluation of protective effects of potential antidotes in this

research like CCl_4 for the screening of possible protective agents.

In the histopathological examination, light microscopy and SEM revealed that the effective dose of TCE administration exhibited no damage on both liver and kidney sections in rats. However, in the TEM, all organelles appeared normal except lipid droplets, which seemed to have increased a little bit. In addition, one important finding noted was the dilation of rER. As ER is also involved in lipid metabolism (Miyai, 1991), the increase of lipid droplets may attribute to the effect of lipid metabolism in rER. As a result, this finding may further conclude that the liver may not be the main target site of TCE toxicity like CCl_4. However, the liver may be affected by TCE to cause minor damage to hepatocytes. It caused a little bit increase in SGPT levels. As a raised activity of SGOT may be due to liver disease, myocardial disease, skeletal muscle disease, renal infarction, haemolysis or hypothyroidism. Based on the present study, the reason for elevation of SGOT may primarily due to the damage of kidney at this point. However, it is still too preliminary to explain whether the actual main target site(s) is/are existed.

Time course study in effective dose of TCE

According to the time course study, the effective dose of TCE causes the significant raise of SGPT and SGOT levels around 6 to 12 hours. The peak value of SGPT and SGOT were seen at 6 hours and 12 hours respectively. Based on this study, both enzyme values seem to have a trend to return back to normal. As SGPT value of TCE treated group could return to normal state at 27 hours later, SGOT value remained slightly higher than that of the vehicle-saline group even at 102 hours later.

However, based on light microscopy, there was no significant pathological change in the sections of liver and kidney in the whole time course. This is probably because the toxicity is expressed only after the capacity to detoxify the chemical of the body has been exceeded (Rouisse & Chakrabarti, 1986). As a result, this study can further conclude that liver and kidney might not be the main target site of TCE because conspicuous enzymes elevation which exceeds the body detoxification system could not cause any significant damage to the organ structure though the significant change in the organs weight at 6 hours after treatment. Especially in the liver, TCE only generated a minor damage to the liver, because the liver is an organ with high power of regeneration (Smuckler, 1975). Thus, SGPT level may return to normal state after certain period. Besides liver and kidney, other organs may be the target sites for TCE to generate acute toxicity.

Curative and preventive tests of seaweed aqueous extracts against the TCE effective dose-induced toxicity.

In this curative test, of the four species of seaweeds under study, aqueous extracts of *S. henslowianum* and *M. myagroides* administered at all three dosages (150 mg/kg, 300 mg/kg and 600 mg/kg) exhibited significant protection against TCE-induced injury in rats by reducing the acute increase of SGPT and SGOT levels. The extract of *S. siliquastrum* also exerted a significant hepatoprotective effect but only at the higher dosages (300 mg/kg and 600 mg/kg). Of these three seaweed species, *S. henslowianum* appeared to be the most promising and effective one. The protective effect of seaweeds in this study was similar to the curative effect in CCl_4 one. As a result, aqueous extracts of seaweeds might contain certain active

component(s) which could relief the TCE-induced toxicity. Based on the SGPT values, the conspicuous reduction in this enzyme might indicate the possible existence of hepatoprotective agent(s) in the extracts. Besides, the active components seemed to protect the actual target site(s) of TCE to return the elevated levels of SGOT back to the normal state. Moreover, the red macroalgae, *Galaxaura* sp. (for preliminary investigation), exhibited a significant curative effect to TCE-induced elevated level of SGPT and SGOT even at the 30 mg/ml saline dosage administered. The effect is comparable with that of the 60 mg/ml saline extracts of *S. henslowianum* and *M. myagroides*. Therefore, *Galaxaura* sp. could also be regarded as an effective agent in this test.

In the liver and kidney weight analysis, no significant change could be seen except those treated with *S. siliquastrum*. Both liver and kidney weights exhibited the significant increase when compared with the TCE toxin control. At this point, *S. siliquastrum* extracts may more or less exert a certain degree of adverse effect to the liver and kidney. However, its actual mechanism of action was still obscure now. This result may be complementary with the one in curative test against CCl_4. As a result, *S. siliquastrum* exhibited a quite inconclusive statement about its action on CCl_4- and TCE-induced toxicity. Therefore, it may act as a negative control in both tests.

In the corresponding preventive test, there were no significant protective effect of *S. henslowianum* and *M. myagroides* extracts to reduce the elevated level of SGPT except that of *S. siliquastrum* extracts and *Galaxaura* sp extract (30 mg/ml saline). Nevertheless, there was a significant action in reducing the SGOT level when each of all seaweed extracts was administered. It showed that the extracts could exert a certain protective effect against the acute elevated level of SGOT. The preventive

effect of those extracts is similar to that of the curative test. Moreover, *S. henslowianum*, *M. myagroides* and *S. siliquastrum* showed more or less similar effect in this preventive test besides the red macroalga, *Galaxaura* sp., which exhibited the most prominent effect even at 30 mg/ml saline when compared with other seaweed extracts at the same dosage. Consequently, it has a potential to exert a good antidotal effect against the TCE-induced toxicity.

Antidotal effects of dimethyl sulfoxide (DMSO) and N-acetylcysteine (NAC) against CCl_4- and TCE-induced poisoning in rats.

Based on the curative and preventive test, it is generally accepted that the curative treatment is better than the preventive treatment. The possible reasons may be due to that DMSO and NAC are easily excreted from the body before the toxin treatment, owing to the presence of sulfide group in their structure. Moreover, they are water-soluble compounds. They may be easily absorbed in and removed out from the body through urination (Klaassen & Rozman, 1991) . As a result, only small amount of residue remains inside the body to achieve the antidotal effect.

According to the results of DMSO and NAC against CCl_4-induced heptotoxicity, post-treatment of DMSO and NAC proved to have a remarkable protective action against chemically induced elevation of SGPT and SGOT level. The same positive effect of protection could also be seen in the organs weight analysis to reduce chemically induced increase of both liver and kidney weights. However, this effect is significant only at the highest dosage. Besides, the protective effect of these two treatments could be confirmed by the histopathological examination of liver cells.

The evidence of scanty necrotic cells accompanied with the presence of wide range of regeneration zone and mitotic figures indicated that DMSO and NAC were active principles to relief the damage of liver. The dose-response relationship can be seen in the histopathological analysis. Especially in the NAC treatment at the higher dosages, significantly reduced SGPT and SGOT levels together with the convincing evidence in histopathological examinations could be shown in CCl_4-induced hepatotoxicity in rats. The result obtained from both biochemical and histopathological tests were quite similar to that of *S. henslowianum* extract treatment against CCl_4-induced hepatotoxicity at the corresponding dosages. The conspicuous recovery of liver cells accompanied with the large range of regeneration zone and scanty necrotic cells were evident of its protective effect. As a result, NAC exerted an excellent protective effect like *S. henslowianum* extract in the curative test. Besides, in the effect of TCE-induced toxicity test, DMSO and NAC in the higher dosages also exhibited the significant protective action to reduce the elevated levels of SGPT and SGOT. Especially in NAC treatment at the highest dosage, the result obtained from the biochemical tests is quite similar to that of *S. henslowianum* extract treatment against TCE-induced toxicity at the corresponding dosage.

DMSO and NAC are important chemical antioxidant (Achudume, 1991; Flanagan & Meredith, 1991; Sies, 1993). The interesting properties of DMSO for its effect to arrest microsomal enzyme activity (Achudume, 1991), and its inhibitory effect on cytochrome P-450-based metabolism (Lind & Gandolfi, 1997), might result in a lack of bioactivation of CCl_4 to its toxic intermediates, CCl_3 ·. According to Siegers (1978), the reduction in paracetamol and bromobenzene-induced liver GSH depletion brought about by DMSO indicates a curb on the formation of conjugates

between glutathione and hepatotoxic metabolites. Therefore, GSH can contribute significantly to the intracellular defense system by its powerful consumer of superoxide, singlet oxygen and hydroxyl radicals (Miesel & Zuber, 1993). Furthermore, NAC can also be used for the synthesis of cysteine and GSH (Sies, 1993). As a result, the hepatoprotective effect of DMSO and NAC can be promising. Based on the above reasons, the possible effect of seaweed extracts may be due to the properties of antioxidant or the scavenging activity against CCl_4- and TCE-induced production of reactive metabolite(s) on the target organ(s). The antidotal effect of DMSO against CCl_4, in the present study, appears to be quite convincing.

There were little information about the DMSO and NAC's protective effect against CCl_4 and TCE-induced toxicity in rat. However, several reports have illustrated the presence of antioxidant properties. The antidotal properties of DMSO may be due to the inhibition of microsomal oxidation to inhibit the reactive metabolites formation to cause the damage (Lind & Gandolfi, 1997) and the regulation of the GSH concentration to protect the liver damage from the reactive metabolites. As the highly reactive metabolites formed from CCl_4 or TCE can combine to the sulfhydryl groups of proteins and inactivation of proteins leads to death of liver cells and, consequently, liver necrosis (Kröger et al., 1997). GSH protects liver proteins because it competes with reactive metabolites for the reaction with the proteins. Although the main target site(s) for TCE in the present study is still obscure, the protective effects of seaweeds, DMSO and NAC were rather ascertain. Based on the protective properties of DMSO and NAC, the possible reactive agent(s) generated from TCE may possibly be free radical which may affect the target site(s) to induce the prominent elevated level of SGOT. Nevertheless, the possible main

target sites may be other organs rather than the liver or kidney.

Hepatoprotective effect of methanol extract of seaweeds against CCl_4- and TCE-induced poisoning in rats

According to the experiment of protective effect of seaweed's methanol extracts, in general, all exhibited significant protective effect in lowering the acute elevated levels of SGPT and SGOT levels induced by CCl_4. The methanol extract of *S. siliquastrum* showed the most prominent protective effect against CCl_4-induced hepatotoxicity. Conversely, the *M. myagroides* extract exhibited the least effect, especially in the SGPT level which is comparable with the 25% DMSO control group. Although the presence of very significant data in reducing the level of SGPT and SGOT, the histopathological examination on *S. siliquastrum* treated liver sections revealed no conspicuous evidence about recovery or large range of regeneration zone when compared with the most effective one, *S. henslowianum*, in the curative test against CCl_4-induced hepatotoxicity. Moreover, the liver and kidney weight showed no significant difference when compared with that of the toxin control and 25% DMSO control groups.

In the curative test against TCE-induced toxicity, in general, all extracts showed significant effect in lowering the SGPT and SGOT levels. The *M. myagroides* extract exhibited very prominent protective effect against the acute elevated levels of SGPT and SGOT. The methanol extracts may contribute different effect or action on the CCl_4 and TCE-induced toxicity. However, they all exert a positive effect in reducing the extent of toxicity induced by both toxins. Based on this primarily test, the

methanol extracts showed a quite conspicuous effect in lowering the acute elevated transaminases activity. However, histopathological examinations did not show any conspicuous and convincing evidence to support the effective effects on enzyme assays, like aqueous extracts in the CCl_4 curative test. Obviously, the methanol extracts are lipid-soluble while aqueous extracts are water-soluble that the active constituents may be different in both extracts. As a result, it may generally conclude that both extracts (aqueous and methanol) contained different types of active ingredient(s) to protect the liver injury induced from CCl_4 in different extent. Furthermore, it must be noted that the methanol extracts of *S. siliquastrum* and *S. henslowianum* at the dosage of 30 mg/ml saline showed the better result than the aqueous extracts of *S. henslowianum* at the same dosage. Therefore, the primary assumption could be drawn that the possible hepatoprotective effect of methanol extracts is better than that of the aqueous ones. The possible component(s) in the methanol extracts may be phenol or polyphenols which are organic in nature (Lee et al., 1996). It is different from the aqueous extracts which are probably polysaccharides or glycoproteins in nature (Harada et al., 1997). Furthermore, the hepatoprotective activity of the extracts was also possibly due to their antioxidant properties, acting as scavengers of free radicals. The curative action on hepatic injury by the methanol extracts protected against liver plasma membrane peroxidative degradation or promoted cellular mitosis action for the repair of the liver cells. Some of these may involve active binding sites. It must be noted, however, that the possible mechanisms of protection are still obscure at this stage and more investigations are needed to clarify the active components of the seaweed's extracts (both aqueous and methanol extract) and study their modes of the hepatoprotective effect in the future study.

Chapter 6 CONCLUSION

Based on the results obtained, several conclusions on the present study can be drawn:

1. According to the preliminary tests on the hepatotoxicity, biochemically and histopathologically in the curative mode, all aqueous seaweed extracts used at their particular dosages contained no hepatotoxic or nephrotoxic agent to cause significant damage to both liver and kidney of the rats.

2. In hepatoprotective screening of seaweed extracts, *Sargassum henslowianum* and *Myagropsis myagroides* have the most effective hepatoprotective effect against the CCl_4-induced toxicity in the curative test. The same result can be demonstrated in the TCE curative test. The extracts of seaweeds are probably associated with (a) their antioxidant properties to act as a free radicals scavenger or antilipoperoxidant activity to remove highly reactive intermediates, (b) their cell division enhancement to promote regeneration or recovery from the damage. The same positive effect can also be shown in the DMSO and NAC treatment.

3. Based on the TCE toxicity test, intraperitoneal route is the most effective way to generate the toxic effect by the elevation of both SGPT and SGOT levels when compared with oral route. The effective toxic dose of TCE has been found to be 20% of TCE in corn oil which is administrated at the dosage of 1.25 ml/kg. This dosage is the same as CCl_4 one. However, it cannot generate the promising effects like CCl_4 in histopathological analysis, according to the histopathological analysis in TEM. The effective dose of TCE can generate

certain toxic effects on the liver with a slight increase of lipid droplets and dilation of rER. However, conspicuous damage like necrosis cannot be found. It indicates that the main target site(s) may attribute to the reason for the SGOT elevation. Moreover, the free radical may be involved to induce the elevation of SGOT level. Further investigation should be followed to identify the main target site(s).

4. The seaweed extracts, DMSO and NAC exhibit a better protective effect in curative mode than that of preventive mode. It indicates the curative mode is more direct than the preventive one. As the antidote treatment is 6 hours before the toxin treatment, the possible action of excretion through urination or other mechanisms may occur to remove all or most of active agent(s) out of the body.

5. In the study of methanol extract of seaweeds, the protective effect can be ascertained by the enzyme assay and histopathological examinations. However, the result of histopathological examination is not as good as that of the curative one even the prominent transaminase activity has been proved.

6. The possible active agents in the methanol extracts are not the same as those in the aqueous extracts. As the methanol extracts are organic in nature to which the presence of phenolic compounds (phenols or polyphenol), organic acid etc. compounds may be possible. The aqueous extracts mainly contain water-soluble compounds, mostly carbohydrate and proteins like polysaccharides and glycoprotein. The actual substance(s) or mechanism which contribute to the protective effect is still speculative now.

These studies represent only the first stages into investigations on the protective properties of potential antidotes against CCl_4 and TCE-induced injury. It must be noted, however, that the possible mechanisms of protection on both toxicity generated from CCl_4 and TCE are rather speculative at this stage and more investigations are needed to identify the active principles of the seaweeds and elucidate their modes of the hepatoprotective action in further studies..

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APPENDIX

Appendix A: **Procedure for preparing a calibration curve to measure the serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activities.**

1. Different volume of the solutions was added as indicated in the table:

Test tube no.	**0**	**1**	**2**	**3**	**4**
Distilled water (ml)	0.1	0.1	0.1	0.1	0.1
Calibration standard solution (ml)*	0	0.05	0.1	0.15	0.2
Substrate (ml)**	0.5	0.45	0.4	0.35	0.3
SGPT activity (IU/L)	0	23	50	83	125
SGOT activity (IU/L)	0	20	55	95	148

2. 0.5 ml color reagent was added to each tube. The tubes were shaken gently and left at room temperature for 20 minutes.
3. 0.4 M sodium hydroxide solution was added to each tube and mixed by inversion.
4. the tubes were left for at least 8 minutes.
5. the absorbance at wavelength 505 nm was read and recorded by using distilled waster as reference.
6. SGPT and SGOT calibration curves of absorbance values versus the corresponding units of SPT and SGOT was plotted.

* Calibration standard solution is 1.5 mM of sodium pyruvate in phosphate buffer of pH 7.5.

** Substrate solution:

0.2 M of DL-aspartate and 1.8 mM of α-ketoglutaric acid for SGOT.

0.2 M of DL-alanine and 1.8 mM of α-ketoglutaric acid for SGPT.

Appendix B: **Procedure for determining the serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activities.**

1. 0.05 ml of serum was added to a test tube.
2. 0.25 ml subrate solution (0.2 M of DL-alanine and 1.8 mM of α-ketoglutaric acid in phosphate buffer of pH 7.5) was added into the test tube containing the serum.
3. the tube was shaken gently and left in water bath for 30 minutes.
4. 0.25 ml of the color reagent (1mM of 2,4-dinitrophenylhydrazine (DNP) in 1 M hydrochloric acid) was added.
5. the test tube was shaken gently and left at room temperature for 20 minutes.
6. 2.5 ml of 0.4 M of sodium hydroxide was added and mixed by inversion.
7. the tube was left for at least 8 minutes.
8. the absorbance at wavelength 505 nm was read and recorded by using distilled waster as reference.
9. SGPT activity was determined from the calibration curve of SGPT.

Appendix C: **Procedure for determining the serum glutamic oxaloacetic transaminase (SGOT) activities.**

1. 0.05 ml of serum was added to a test tube.
2. 0.25 ml subrate solution (0.2 M of DL-aspartate and 1.8 mM of α-ketoglutaric acid in phosphate buffer of pH 7.5) was added into the test tube containing the serum.
3. the tube was shaken gently and left in water bath for 30 minutes.
4. 0.25 ml of the color reagent (1mM of 2,4-dinitrophenylhydrazine (DNP) in 1 M hydrochloric acid) was added.
5. the test tube was shaken gently and left at room temperature for 20 minutes.
6. 2.5 ml of 0.4 M sodium hydroxide was added and mixed by inversion.
7. the tube was left for at least 8 minutes.
8. the absorbance at wavelength 505 nm was read and recorded by using distilled waster as reference.
9. SGOT activity was determined from the calibration curve of SGOT.

Appendix D: **Tissue Preparation Procedure for Light Microscopy** (sample thickness is 5-7 nm)

PROCEDURE	REAGENTS	TIME
Fixing	Bouin' s Fluid	~24 hours
Processing	***Dehydration:***	
	50% Ethanol	1 hour
	70% Ethanol	1 hour
	85% Ethanol	2 hours
	95% Ethanol	2 hours
	100% Ethanol	1 hour
	100% Ethanol	1 hour
	100% Ethanol	1 hour
	Clearing:	
	Xylene: Ethanol (1:1)	1/2 hour
	Xylene	1/2 hour
	Xylene	1/2 hour
	Infiltration:	
	Paraffin wax	1/2 hour
	Paraffin wax	1/2 hour
	Paraffin wax (Vacuum aspiration if necessary)	1/2 hour
	Embedding:	
	Paraffin wax (allow to cool and trim)	
Sectioning	Section at 5 μm thick, adhere on slides by egg albumin and dry on warm plate or oven overnight	
Staining	***Dewax:***	
	Xylene	5 min
	Xylene	5 min
	Hydration:	
	100% Ethanol	1 min
	95% Ethanol	1 min
	70% Ethanol	1 min
	50% Ethanol	1 min
	30% Ethanol	1 min
	running tap water	1 min

	Staining:	
	Mayer' s Hematoxylin	~20 min
	tap water	1 min
	1% Acid Alcohol	~5 sec
	tap water	1 min
	Scott' s tap water	2 min
	tap water	1 min
	0.5% aqueous Eosin	3 min
	tap water	1 min
	Dehydration:	
	70% Ethanol	short/fast
	95% Ethanol	short/fast
	100% Ethanol	2 min
	100% Ethanol	2 min
	Xylene:Ethanol	2 min
	Xylene	2 min
	Xylene	2 min
	Mounting:	-
	Canada Balsam	

Appendix E: **Tissue Preparation Procedure for TEM**

PROCEDURE	REAGENT	TIME	TEMP.	REMARKS
Fixation	2.5% glutaraldehyde in phosphate buffer (mince specimen into 1 mm cubes with a sharp blade after 1-2 hour fixation)	4 hours	4 °C	
buffer Wash	Phosphate buffer Phosphate buffer	5 - 10 mins 5 - 10 mins	R. T. R. T.	
Post-fixation	1% osmium tetroxide in phosphate buffer	2 hours	R. T.	
buffer wash	Phosphate buffer Phosphate buffer	5 - 10 mins 5 - 10 mins	R. T. R. T.	
Dehydration	50% ETOH 70% ETOH 85% ETOH 95% ETOH 95% ETOH 100% ETOH 100% ETOH 100% ETOH	5 - 10 mins 5 - 10 mins 5 - 10 mins 5 - 10 mins 10 - 15 mins 10 - 15 mins 10 - 15 mins 10 - 15 mins	R. T. R. T. R. T. R. T. R. T. R. T. R. T. R. T.	volume of reagent required ~ 3 ml / vial
Infiltration	100% ETOH: Pure spurr (2 : 1) 100% ETOH: Pure spurr (1 : 1) 100% ETOH: Pure spurr (1 : 2) Pure spurr Pure spurr Pure spurr	1 - 4 hours 1 - 4 hours Overnight 2 - 3 hours 2 - 3 hours 2 - 3 hours	R. T. R. T. R. T. R. T. R. T. R. T.	4.5 ml Spurr / vial 10 ml Spurr / vial
Embedding	Pure spurr	8 - 16 hours	68 °C	~ 0.3 ml Spurr / block

Appendix F: **Tissue Preparation Procedure for SEM**

PROCEDURE	REAGENT	TIME	TEMP.	REMARKS
Fixation	2.5% glutaraldehyde in phosphate buffer	~2 - 24 hours	R. T.	- Glutaraldehyde solution prepared can be kept at 4 °C for no longer than 2 weeks
buffer Wash	Phosphate buffer Phosphate buffer	5 - 10 mins 5 - 10 mins	R. T. R. T.	
Post-fixation	1% osmium tetroxide in phosphate buffer	1 - 2 hours	R. T.	- To prepare OsO_4, add PB and stand O/N at R. T. for complete dissolution - OsO_4 must be kept in the dark in -20 °C freezer - Discard OsO_4 if it turns from pale yellow to colorless
buffer wash	Phosphate buffer Phosphate buffer	5 - 10 mins 5 - 10 mins	R. T. R. T.	
Dehydration	50% ETOH 70% ETOH 85% ETOH 95% ETOH 95% ETOH 100% ETOH 100% ETOH 100% ETOH	15 mins 15 mins 15 mins 15 mins 15 mins 15 mins 15 mins 15 mins	R. T. R. T. R. T. R. T. R. T. R. T. R. T. R. T.	
	Critical Point drying (liquid CO_2)			
Coating	Coat the specimen with gold / palladium by sputter coater			

Appendix G: **Reagent preparation for TEM**

1. Phosphate buffer (0.1 M, pH 7.2)

(a) Solution A stock

Na_2HPO_4 (anhydrous form): 7.10 gm / 250 ml

(b) Solution B stock

$NaH_2PO_4.2H_2O$: 3.90 gm / 125 ml

Mix 2 stock solution as follows: (Storage of buffer is not recommended because of microbial growth)

Solution A (ml)	Solution B (ml)	Distilled water (ml)	Final volume (ml)
36	14	50	100
72	28	100	200
108	42	150	300

2. Methanolic uranyl stain

(a) weigh out 0.25 gm uranyl acetate into a 25 ml volumetric flask

(b) add ~ 20 ml methanol to the flask, and shake until completely dissolved

(c) add methanol to the 25 ml mark

(d) filter with millipore filter

(e) aliquot to 0.75 ml / eppendorf tube

(f) store at 4 °C in the dark (remain effective for at least 1 month)

(g) discard stain if it turns cloudy

3. Reynold's lead citrate stain

(a) weigh out 2.01 gm sodium citrate.$2H_2O$ and 1.33 gm lead nitrate, and add 30 ml of fresh distilled H_2O to a 50 ml volumetric flask

(b) shake thoroughly for 1 min and intermittently for 30 mins

(c) add 8.0 ml 1 M NaOH and shake until completely dissolved

(d) add distilled H_2O to the 50 ml mark

(e) filter with millipore filter

(f) aliquot to 0.75 ml / eppendorf tube

(g) freshly prepare the 1 M NaOH (1.0 gm NaOH / 25 ml)

(h) store in an air-tight container (remain effective for up to 6 months)

(i) discard stain if it turn cloudy

Appendix H: **Reagent preparation for SEM**

1. Phosphate buffer (0.1 M, pH 7.2)

(a) Solution A stock

Na_2HPO_4 (anhydrous form): 7.10 gm / 250 ml)

(b) Solution B stock

$NaH_2O_4.2H_2O$: 3.90 gm / 125 ml

Mix 2 stock solutions as follows: (storage of buffer in not recommended because of microbial growth)

Solution A (ml)	Solution B (ml)	Distilled water (ml)	Final volume (ml)
36	14	50	100
72	28	100	200
108	42	150	300

Appendix I: The Procedure for film development and photo printing

1. Kodak TMX 100 Film (135 or 120) Development (in darkness)

PROCEDURE	DILUTION (STOCK : WATER)	TEMPERATURE (^{O}C)	TIME
Running tap water	---	---	Twice
Kodar D76 developer	1 : 1	20	12 mins (shake gently)
Running tap water	---	---	Twice
Iiford Hypam rapid fixer	1 : 4 (reusable for 3 times)	20	2 mins (shake gently)
Running tap water	---	---	At least 15 mins

2. Kodak TEM Film Development (under safe light)

PROCEDURE	DILUTION (STOCK : WATER)	TEMPERATURE (^{O}C)	TIME
Kodar D19 developer	2 : 3	20	4 mins (shake gently)
Running tap water	---	---	2 mins
Iiford Hypam rapid fixer	1 : 4 (reusable for 3 times)	20	2 mins (shake gently)
Running tap water	---	---	At least 15 mins

3. Photo Printing (under safe light)

PROCEDURE	DILUTION (STOCK : WATER)	TEMPERATURE (^{O}C)	TIME
Multigrade IIfospeed paper developer	1 : 9 (use with 1 day)	20	1 min
Stop bath	1 : 19 (use 28% acetic acid stock)	20	5 secs
IIford Hypam rapid fixer	1 : 9	20	1 mins
Running tap water	---	---	At least 15 mins

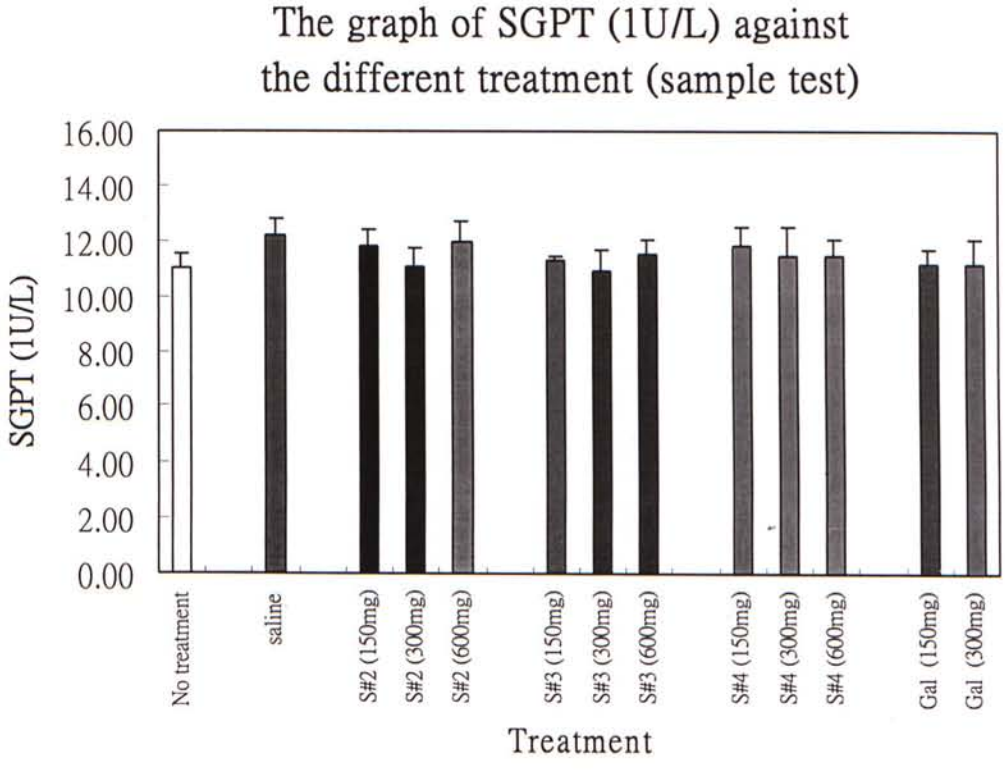


Figure 4.1: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg except *Galaxaura* sp. at dosages of 150 mg/kg and 300 mg/kg) of three species of seaweeds on the level of SGPT activity. Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of vehicle-saline control group are not noted.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*, Gal= *Galaxaura* sp.

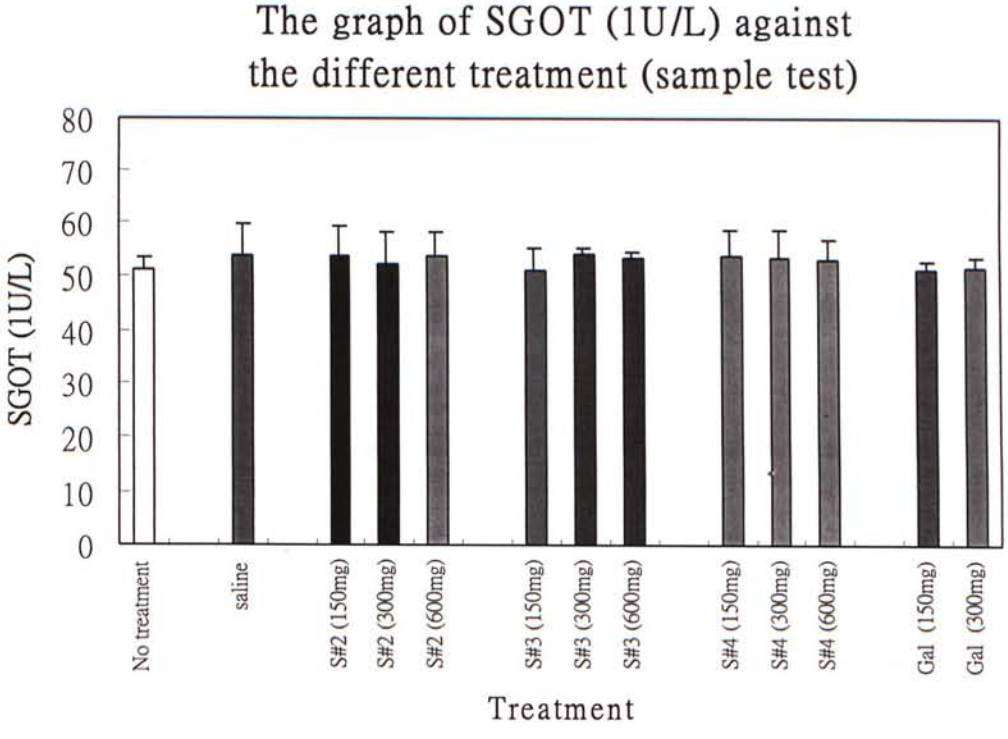


Figure 4.2: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg except *Galaxaura* sp. at dosages of 150 mg/kg and 300 mg/kg) of three species of seaweeds on the level of SGOT activity. Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of vehicle-saline control group are not noted.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*, Gal= *Galaxaura* sp.

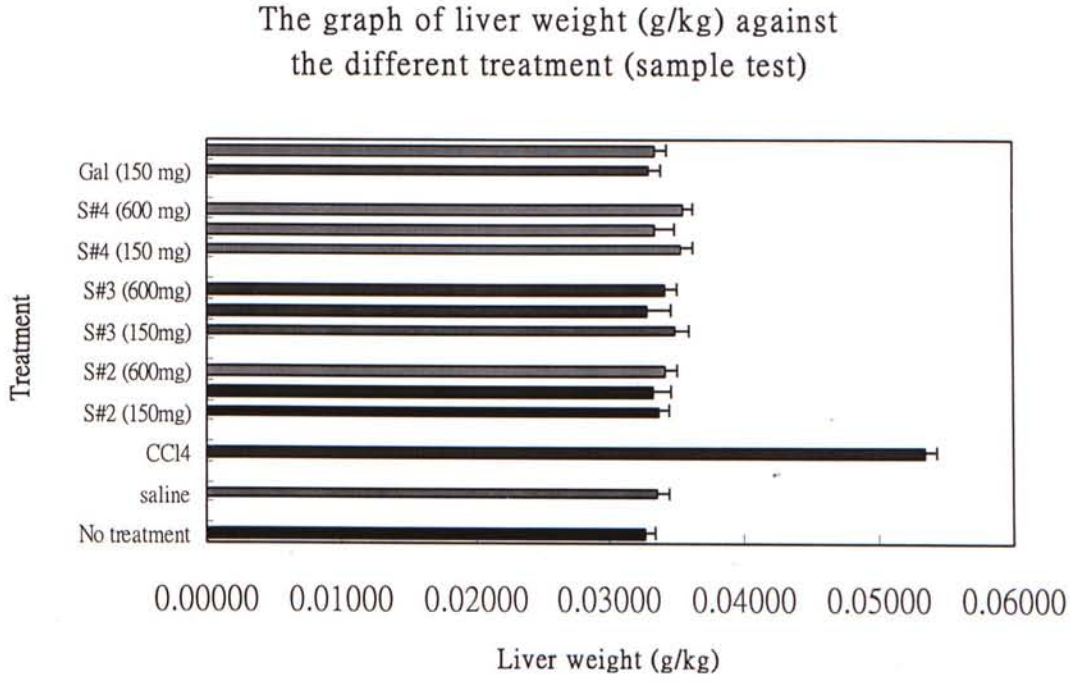


Figure 4.3: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg except *Galaxaura* sp. at dosages of 150 mg/kg and 300 mg/kg) of three species of seaweeds on effect of liver weight. Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of vehicle-saline control group are not noted.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*, Gal= *Galaxaura* sp.

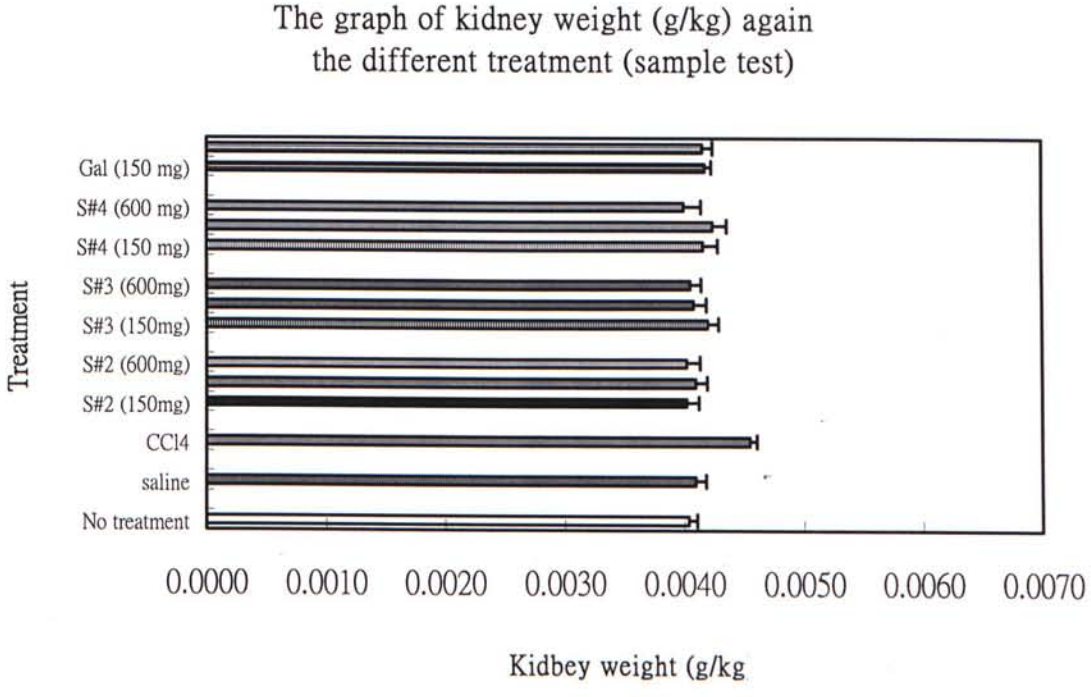


Figure 4.4: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg except *Galaxaura* sp. at dosages of 150 mg/kg and 300 mg/kg) of three species of seaweeds on effect of kidney weight. Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of vehicle-saline control group are not noted.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*, Gal= *Galaxaura* sp.

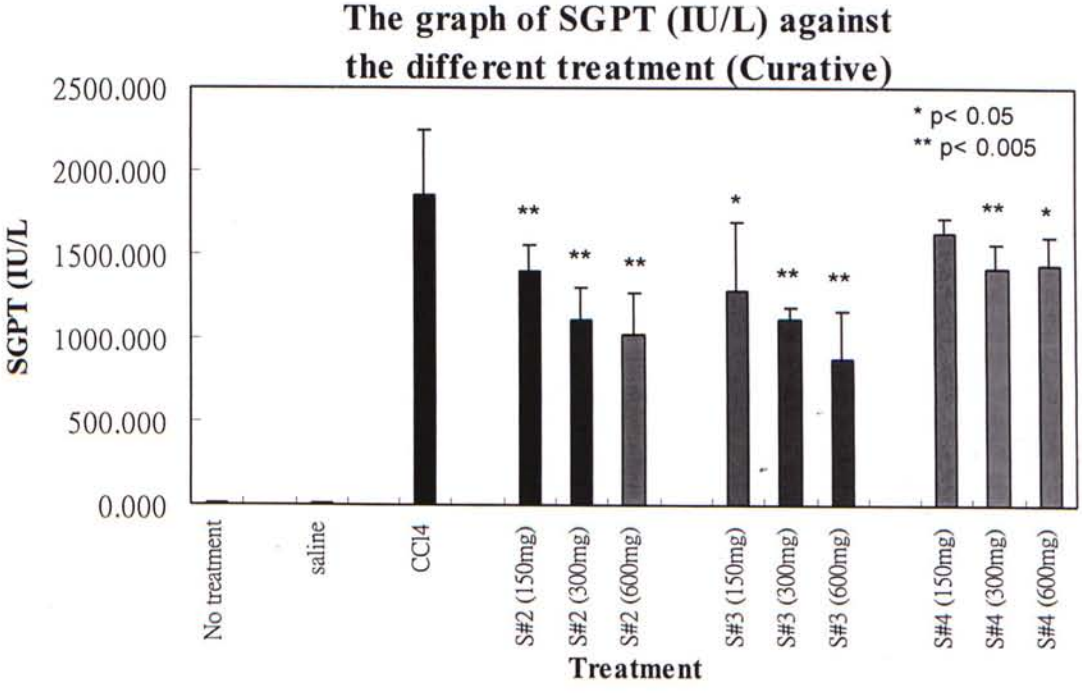


Figure 4.5: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg) of three species of seaweeds on CCl_4-induced elevation of SGPT activity (Curative). Each value represents the mean±S.E.M. of 10 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

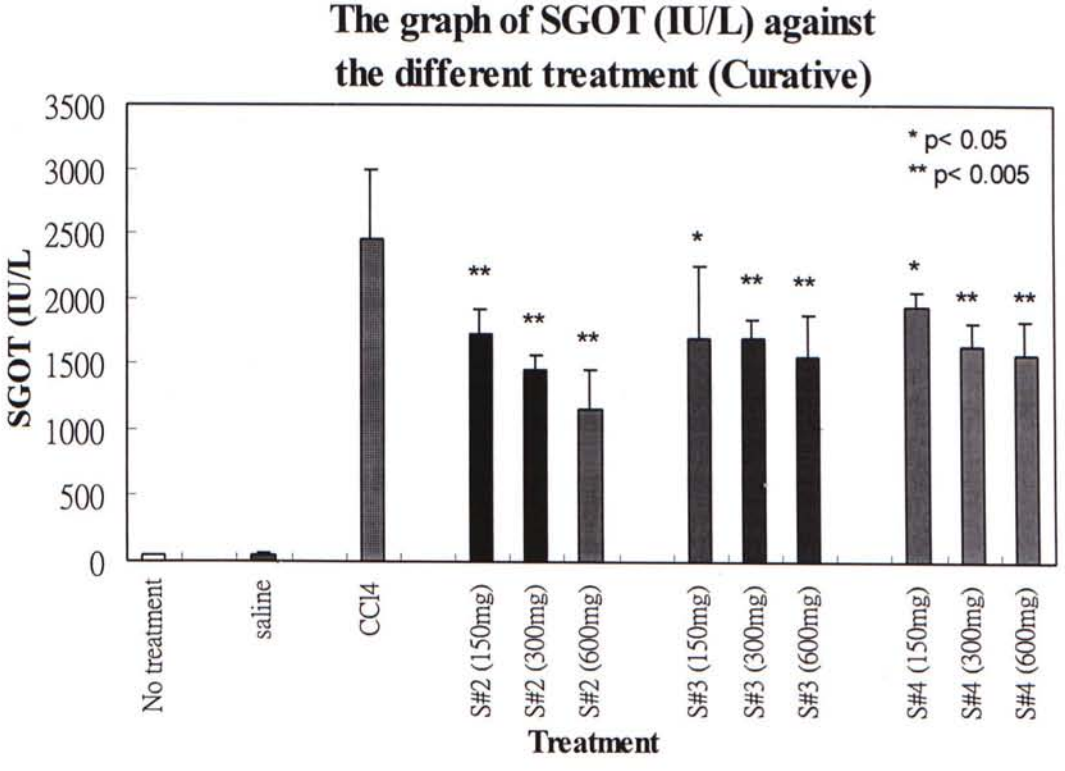


Figure 4.6: Effect of extract (at dosages of 150 mg/kg, 300 mg/ kg and 600 mg/ kg) of three species of seaweeds on CCl_4-induced elevation of SGOT activity (Curative). Each value represents the mean±S.E.M. of 10 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

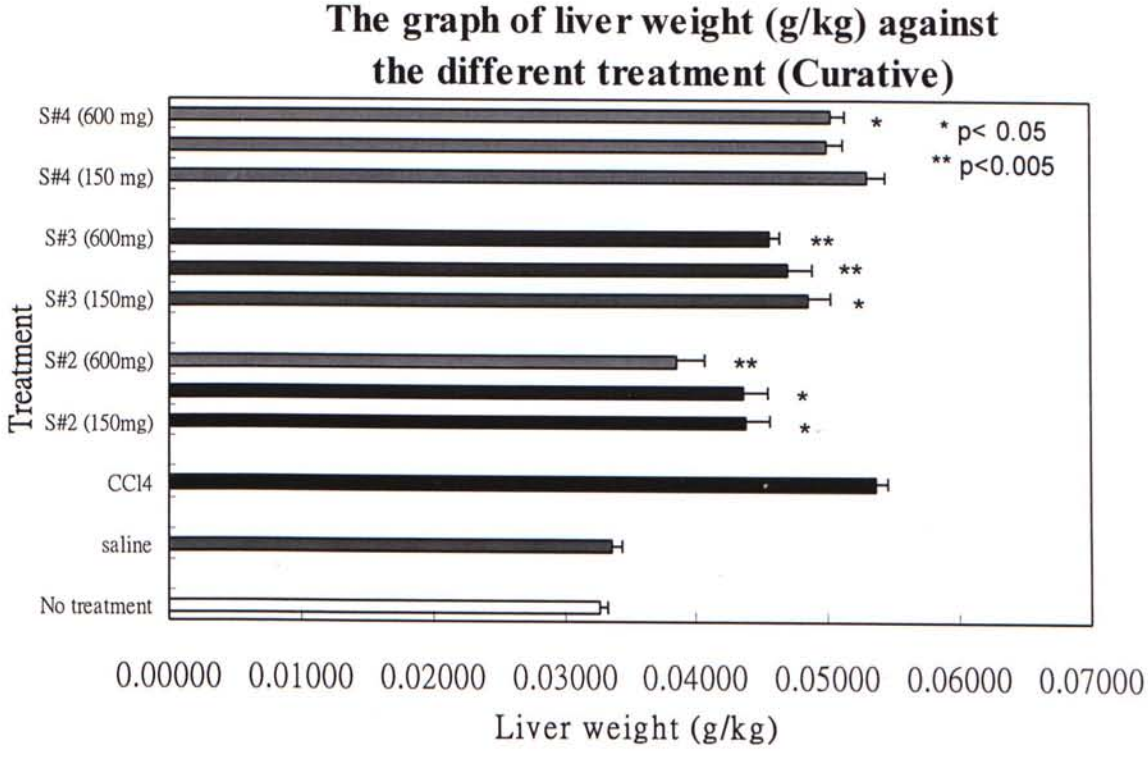


Figure 4.7: Effect of extract (at dosages of 150 mg/kg, 300 mg/ kg and 600 mg/ kg) of three species of seaweeds on CCl_4-induced increase of liver weight (Curative). Each value represents the mean±S.E.M. of 10 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

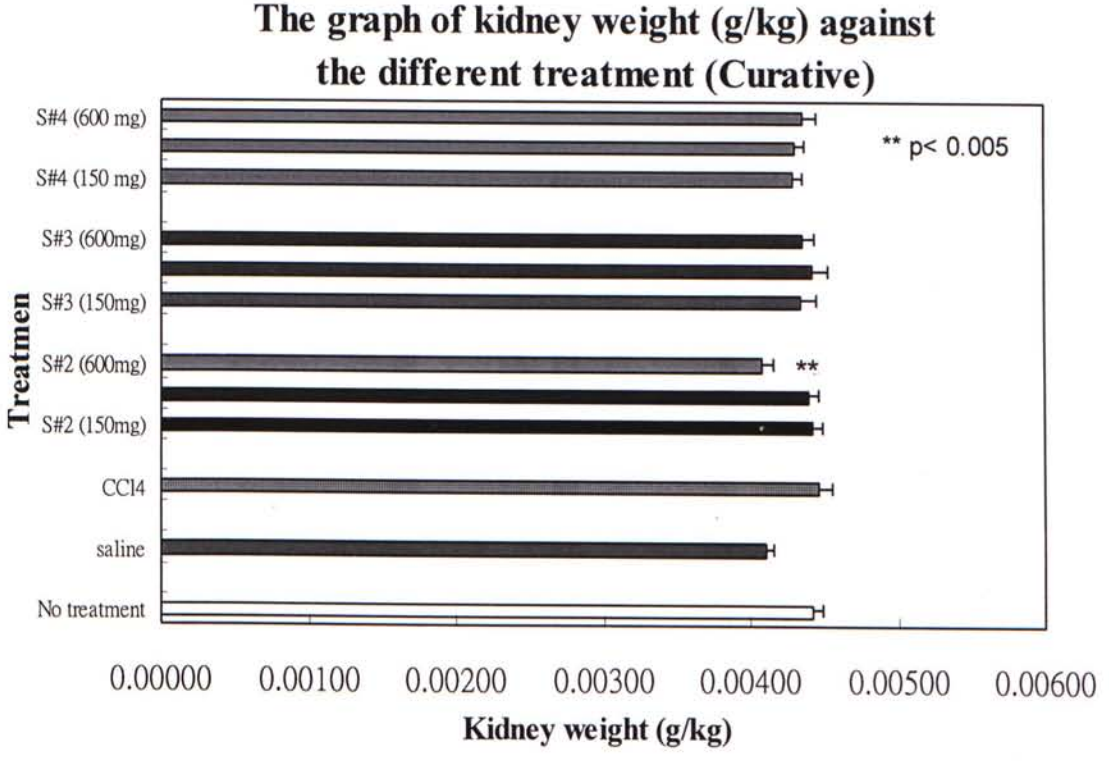


Figure 4.8: Effect of extract (at dosages of 150 mg/kg, 300 mg/ kg and 600 mg/ kg) of three species of seaweeds on CCl_4-induced increase of kidney weight (Curative). Each value represents the mean±S.E.M. of 10 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

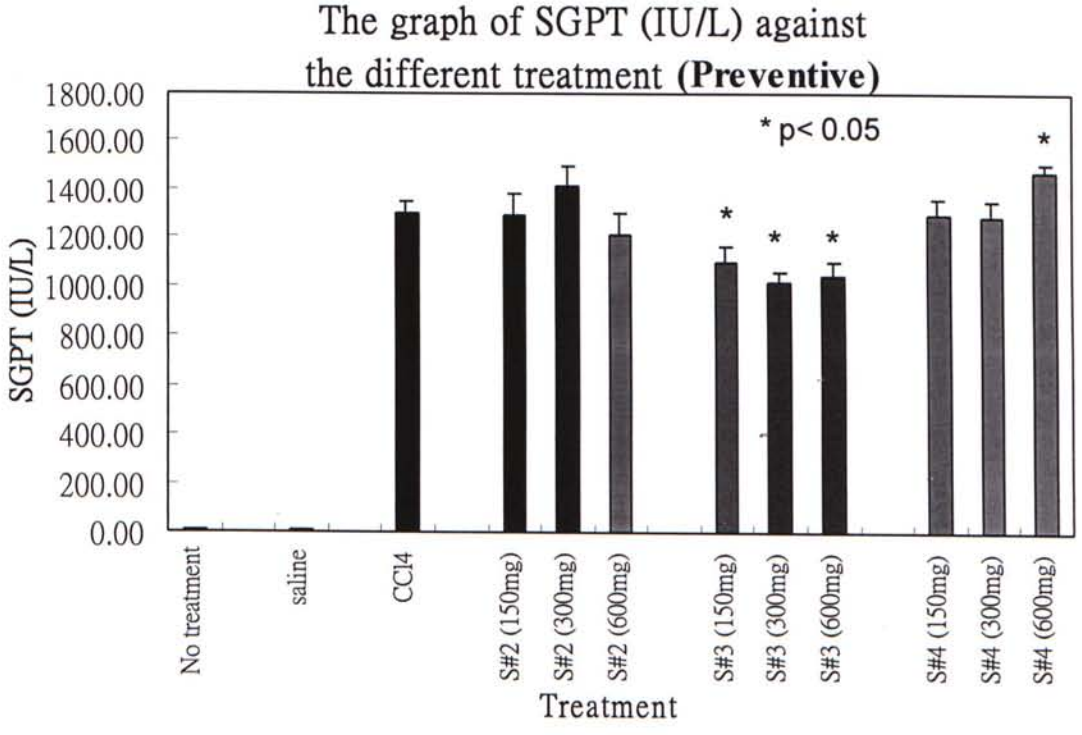


Figure 4.9: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg) of three species of seaweeds on CCl_4-induced elevation of SGPT activity (Preventive). Each value represents the mean±S.E.M. of 7 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

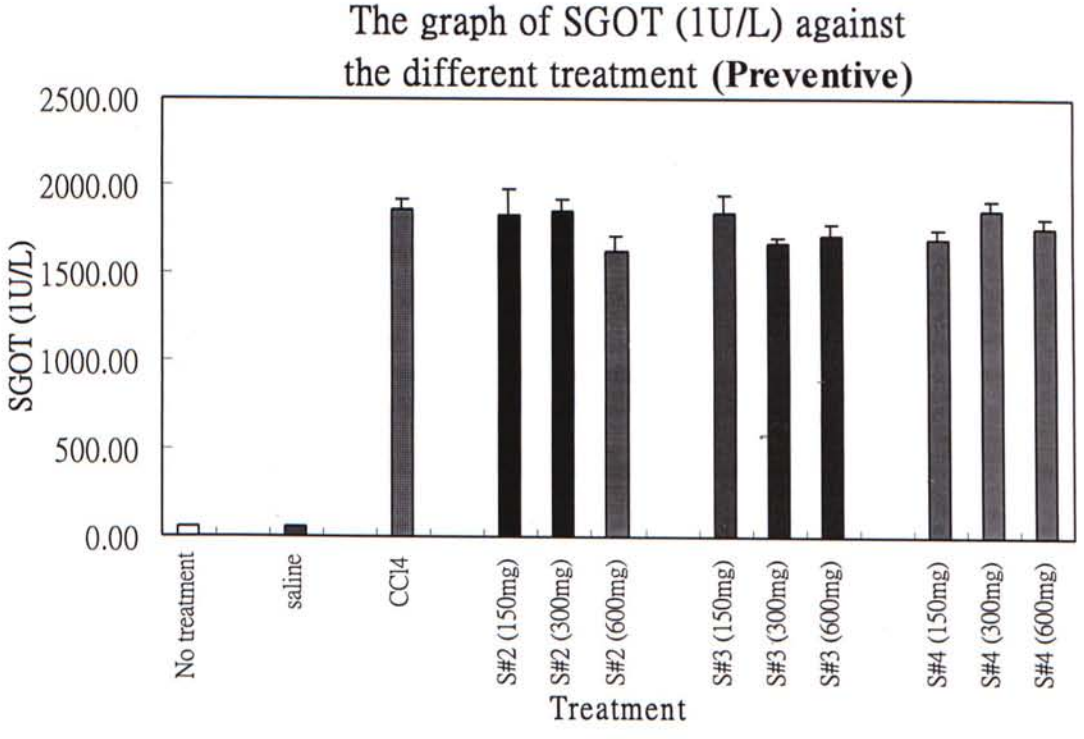


Figure 4.10: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg) of three species of seaweeds on CCl_4-induced elevation of SGOT activity (Preventive). Each value represents the mean±S.E.M. of 7 treated rats. Values statistically significantly different from that of toxin control group are not noted.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastru*

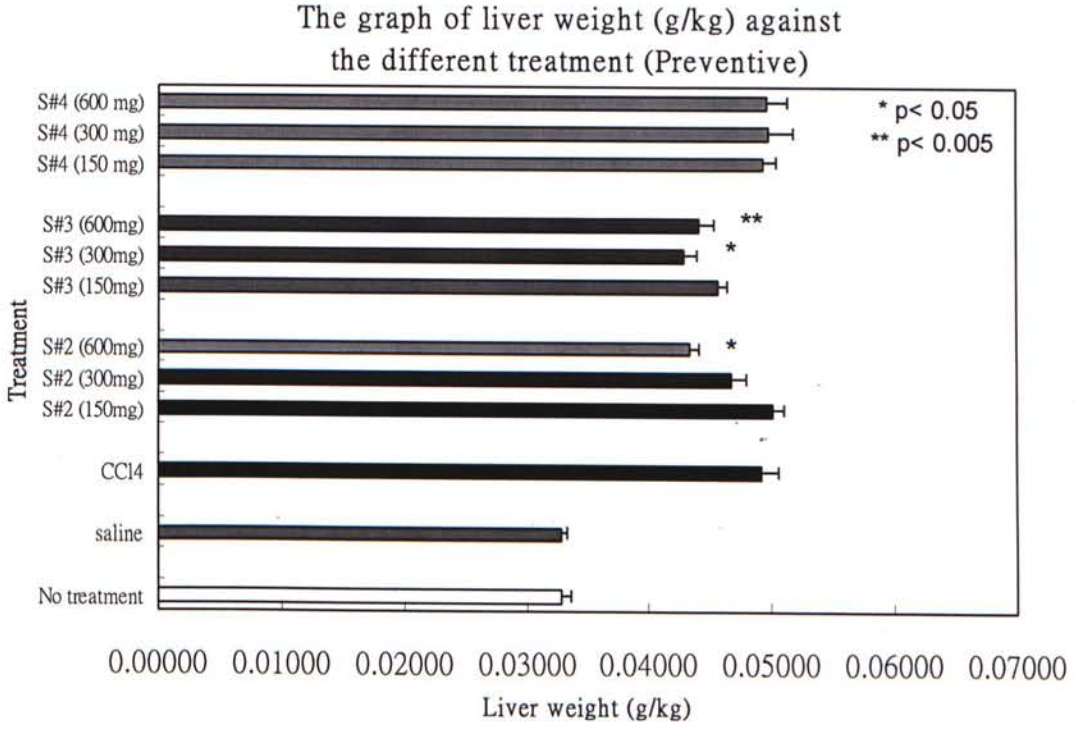


Figure 4.11: Effect of extract (at dosages of 150 mg/kg, 300 mg/ kg and 600 mg/ kg) of three species of seaweeds on CCl_4-induced increase of liver weight (Preventive). Each value represents the mean±S.E.M. of 7 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

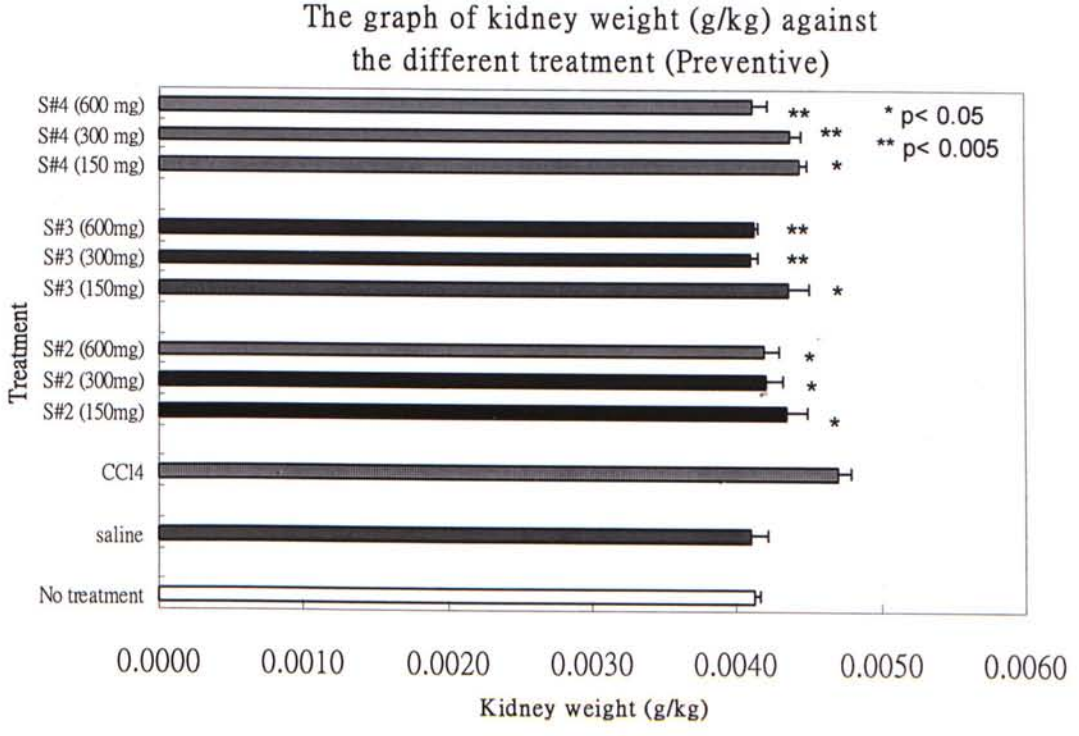


Figure 4.12: Effect of extract (at dosages of 150 mg/kg, 300 mg/ kg and 600 mg/ kg) of three species of seaweeds on CCl_4-induced increase of kidney weight (Preventive). Each value represents the mean±S.E.M. of 7 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

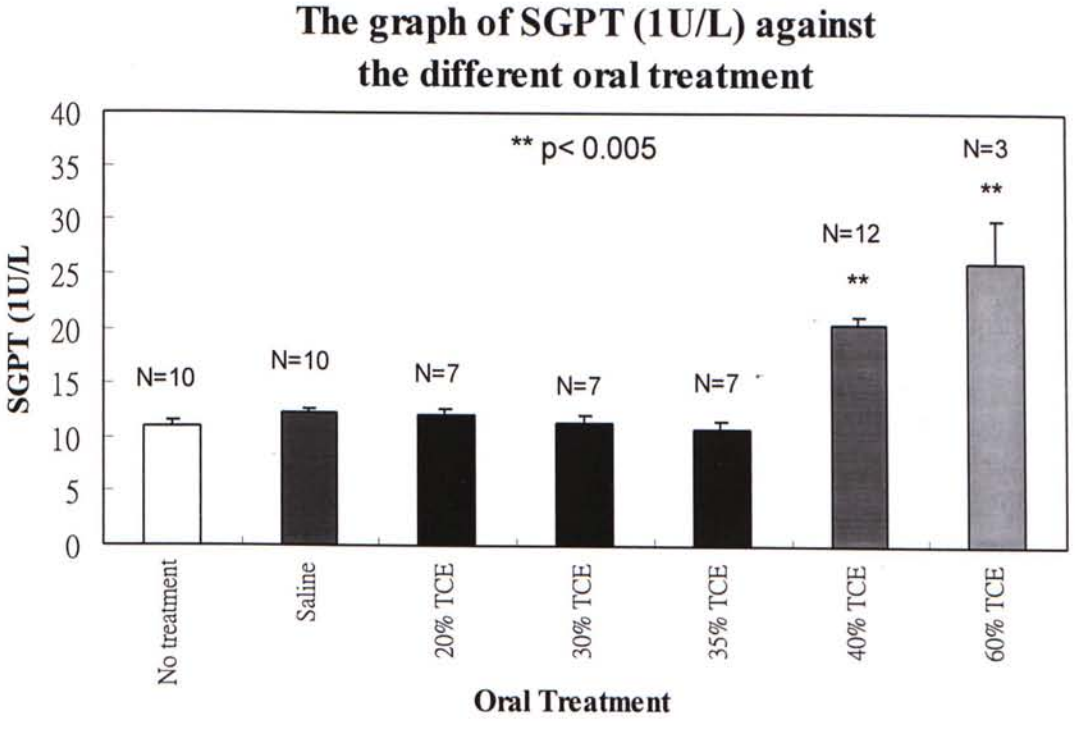


Figure 4.13: Effect on SGPT, one-time oral dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

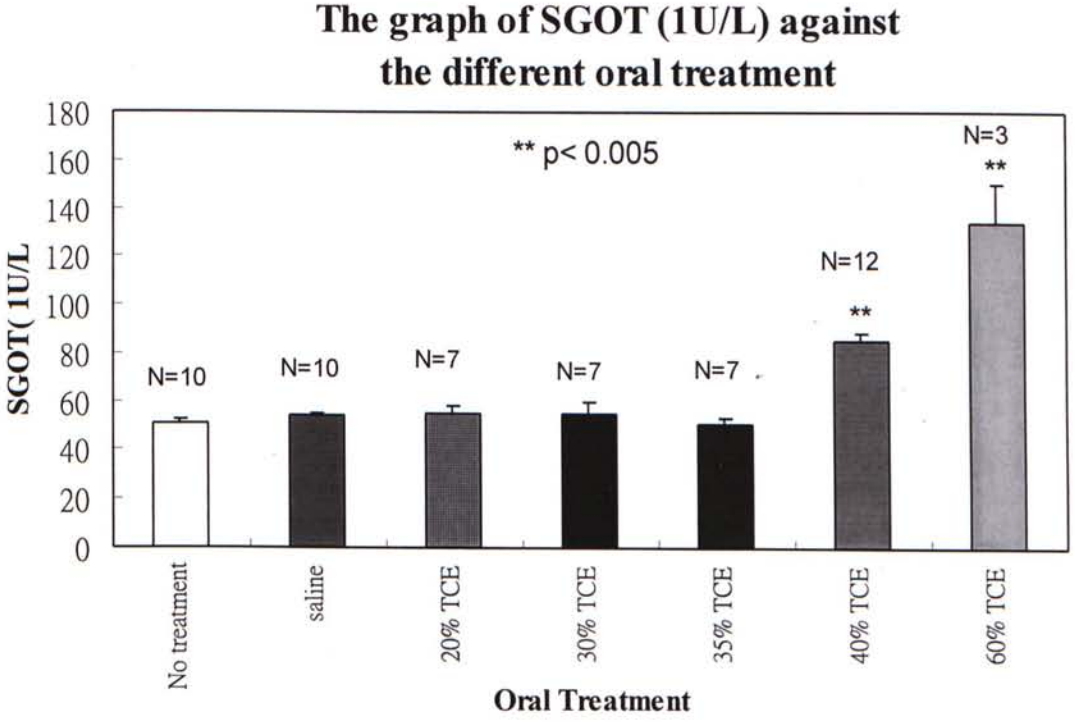


Figure 4.14: Effect on SGOT, one-time oral dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

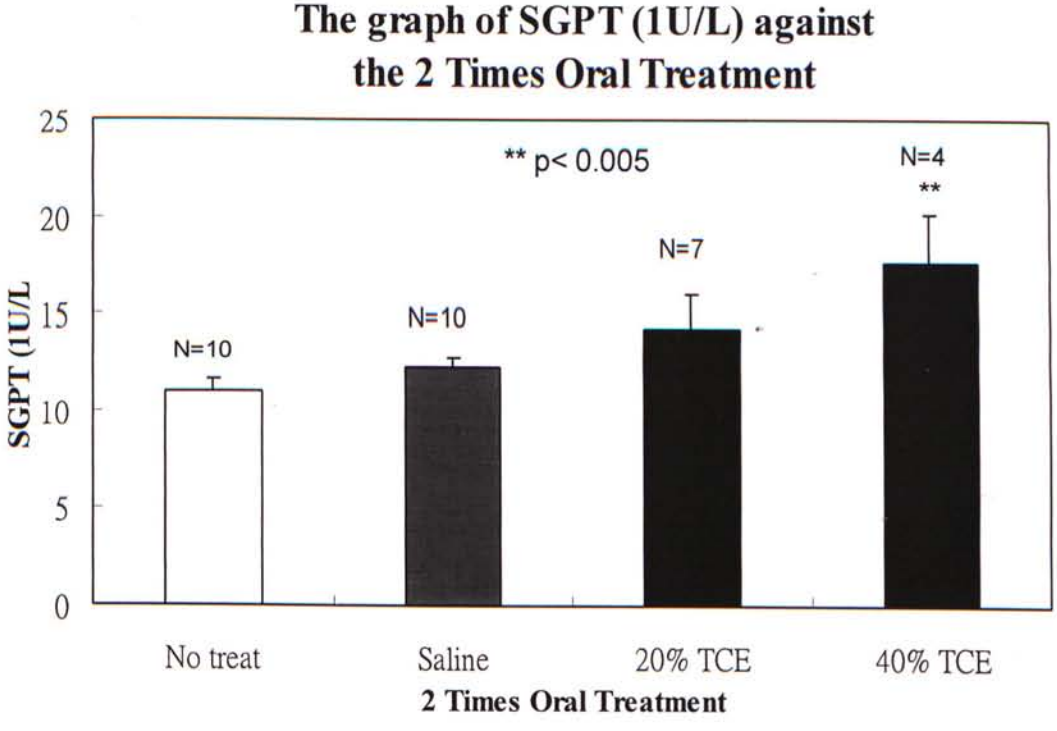


Figure 4.15: Effect on SGPT, two-time oral dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

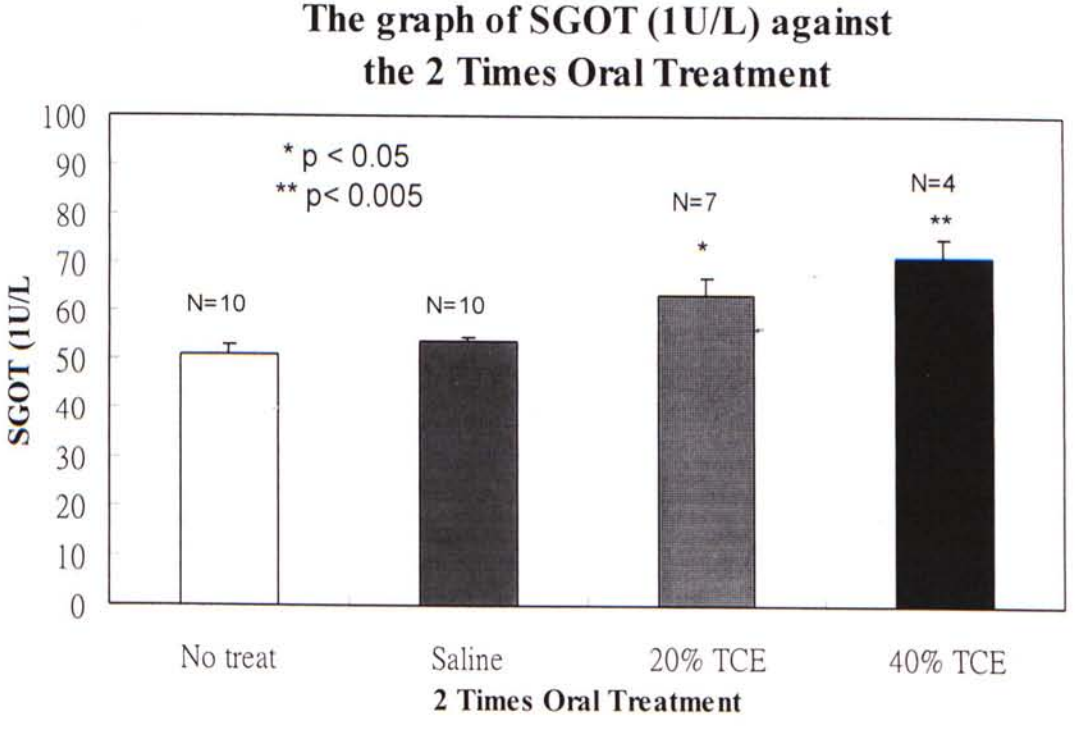


Figure 4.16: Effect on SGOT, two-time oral dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

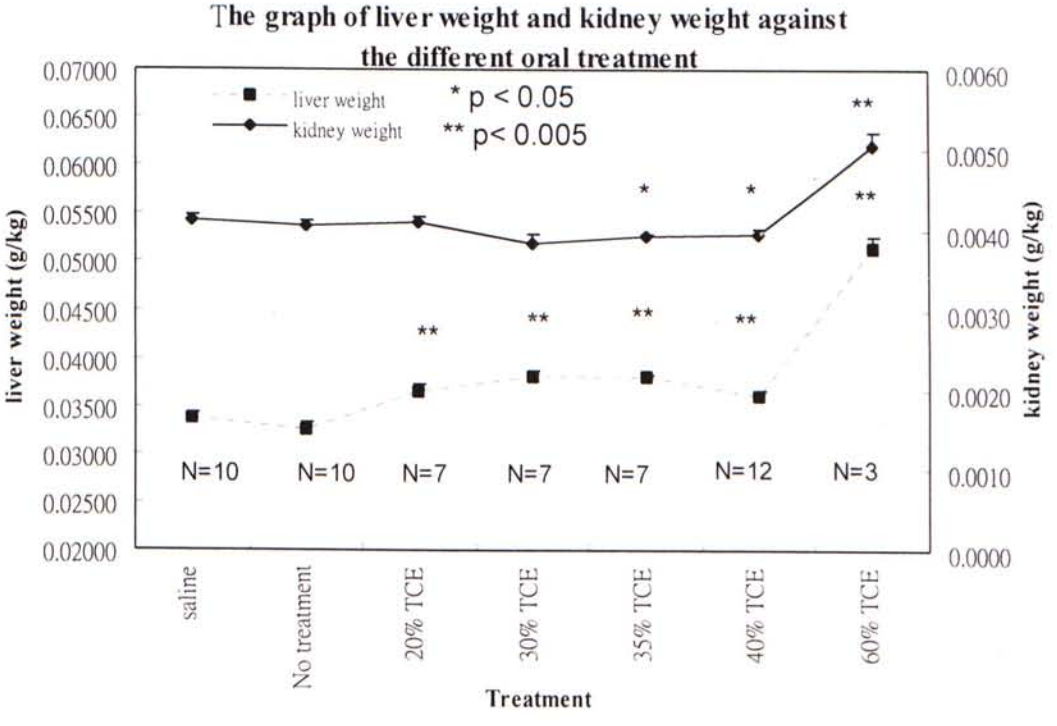


Figure 4.17: Effect on liver and kidney weights, one-time oral dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

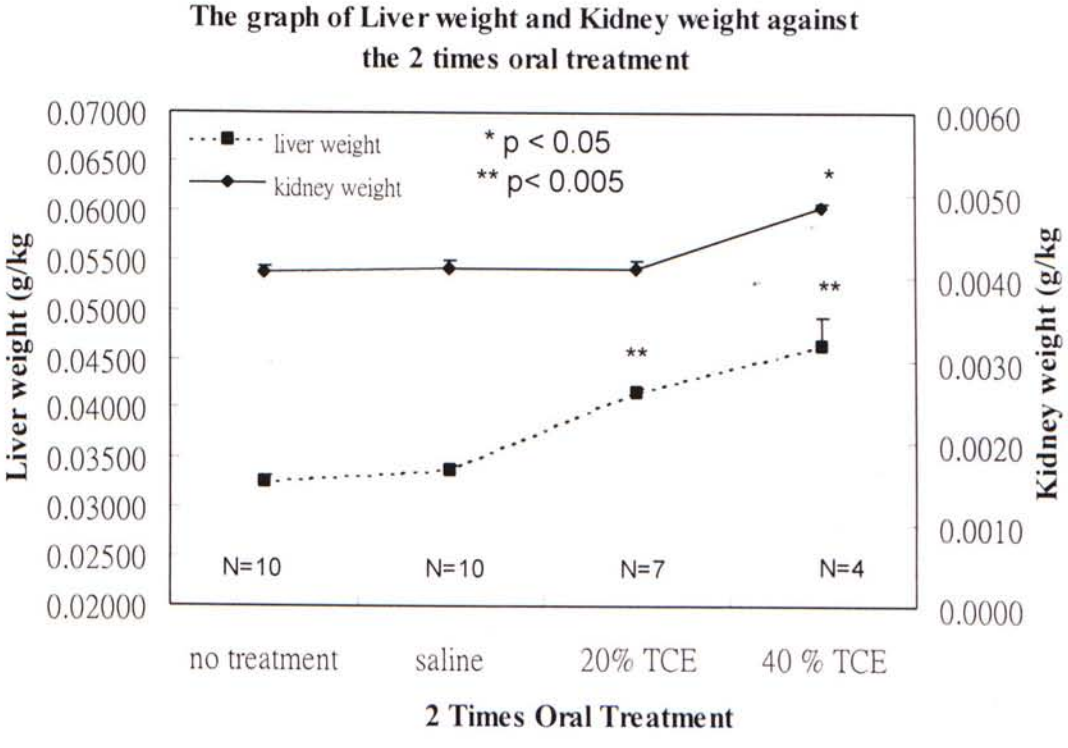


Figure 4.18: Effect on liver and kidney weights, two-time oral dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

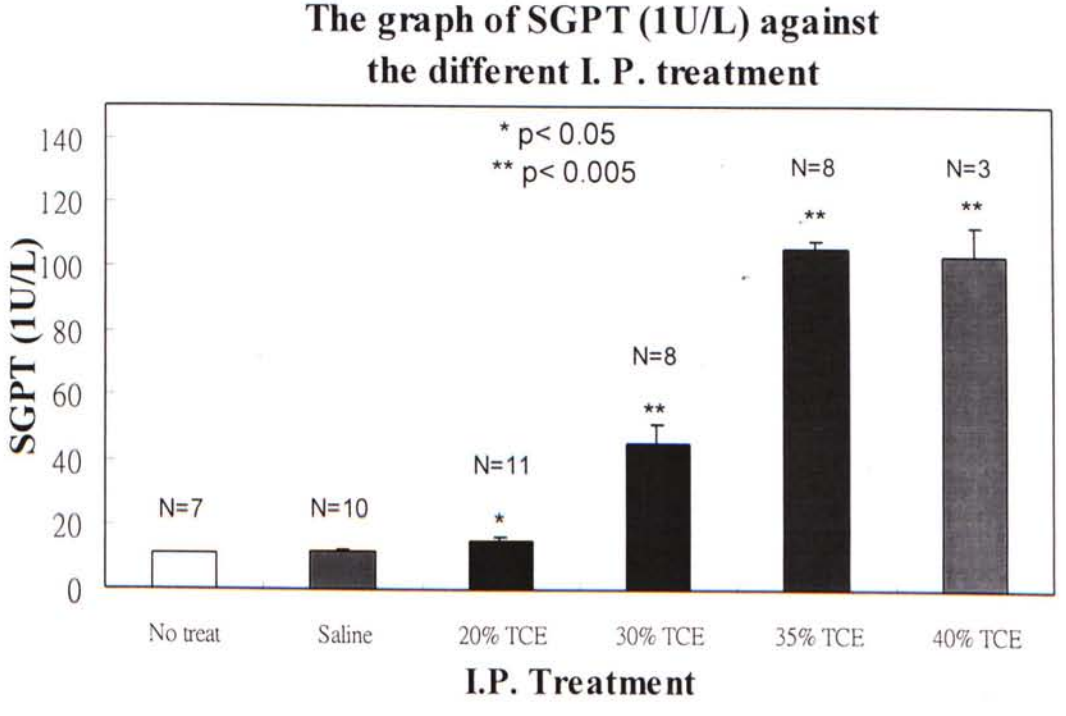


Figure 4.19: Effect on SGPT, i.p. dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

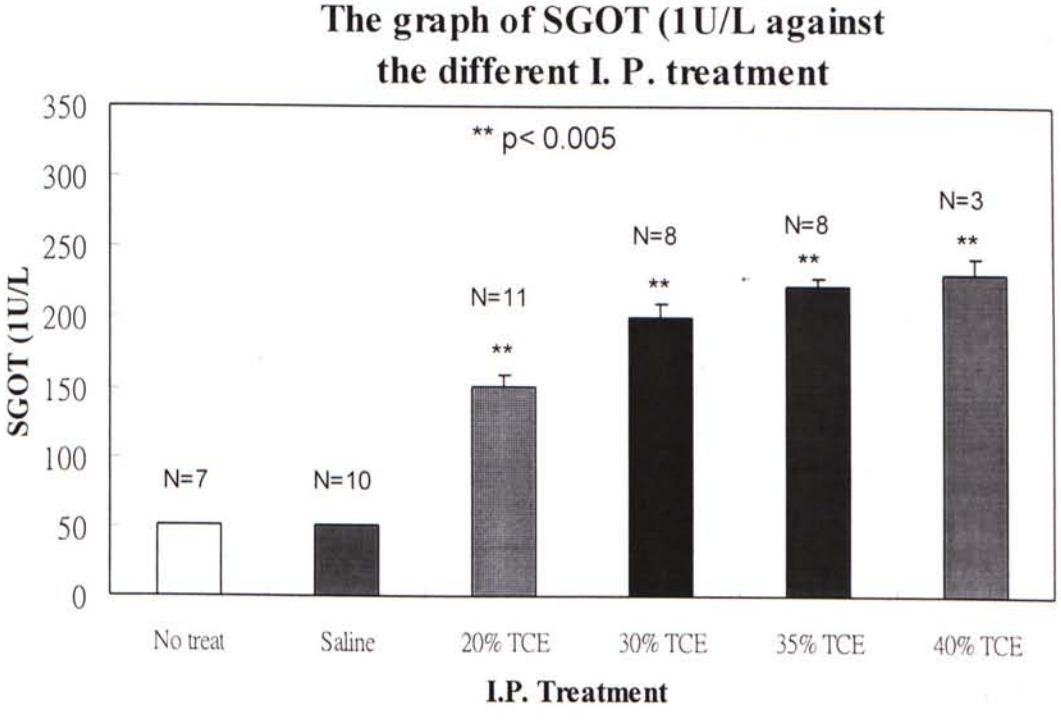


Figure 4.20: Effect on SGOT, i.p. dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

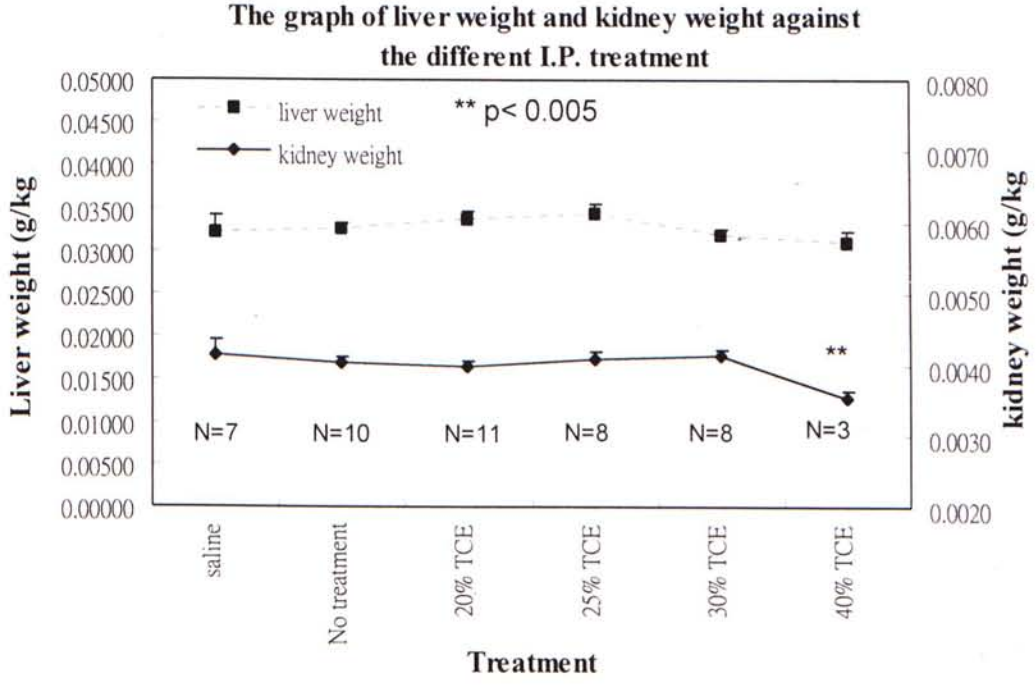


Figure 4.21: Effect on liver and kidney weights, i.p. dosage of TCE in different percentages applied. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group are shown.

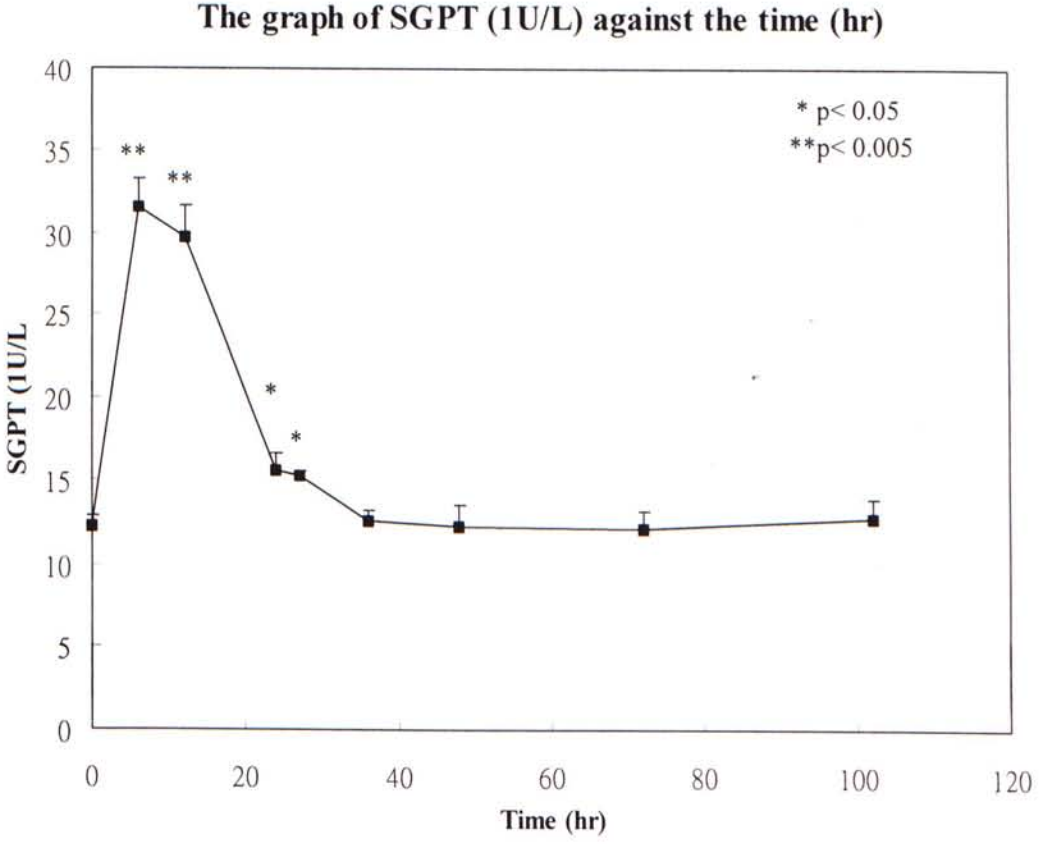


Figure 4.22: Effect on SGPT, i.p. effective dose of 20% TCE in different times (Time course). Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group (0 h) are shown.

0 h: (N=7); 6 h: (N=8); 12 h: (N=7); 24 h: (N=11); 27 to 102 h: (N=6)

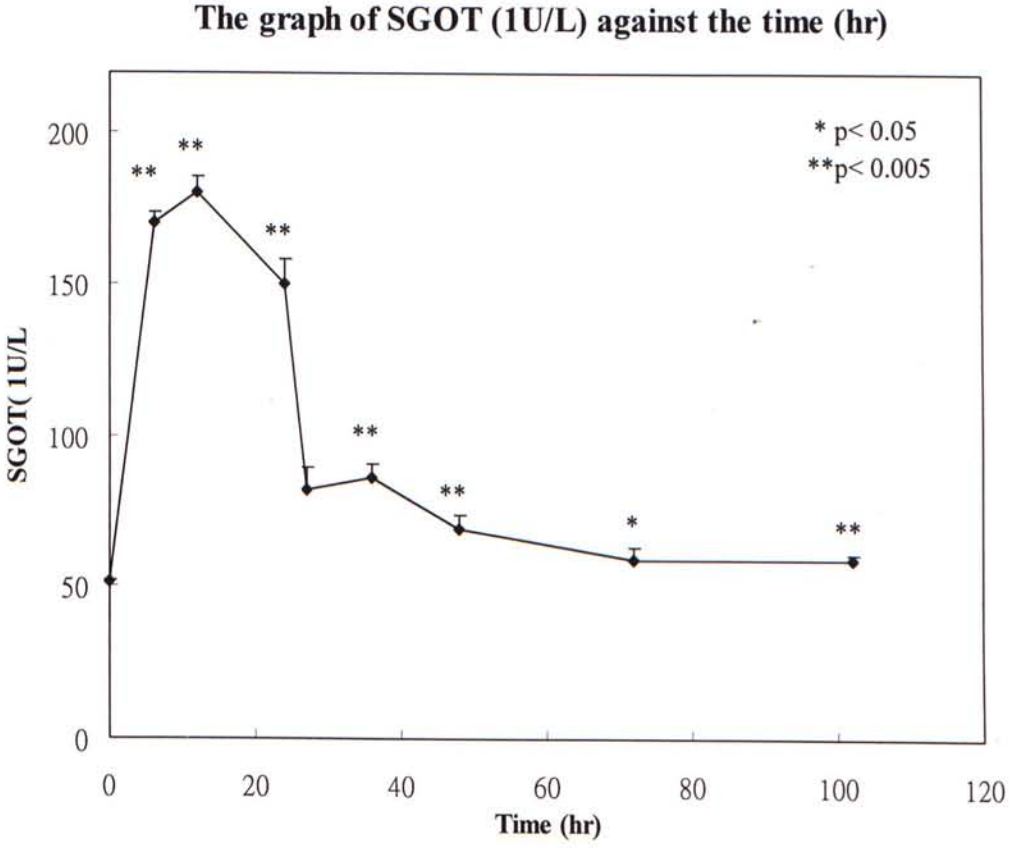


Figure 4.23: Effect on SGOT, i.p. effective dose of 20% TCE in different times (Time course).. Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group (0 h) are shown.

0 h: (N=7); 6 h: (N=8); 12 h: (N=7); 24 h: (N=11); 27 to 102 h: (N=6)

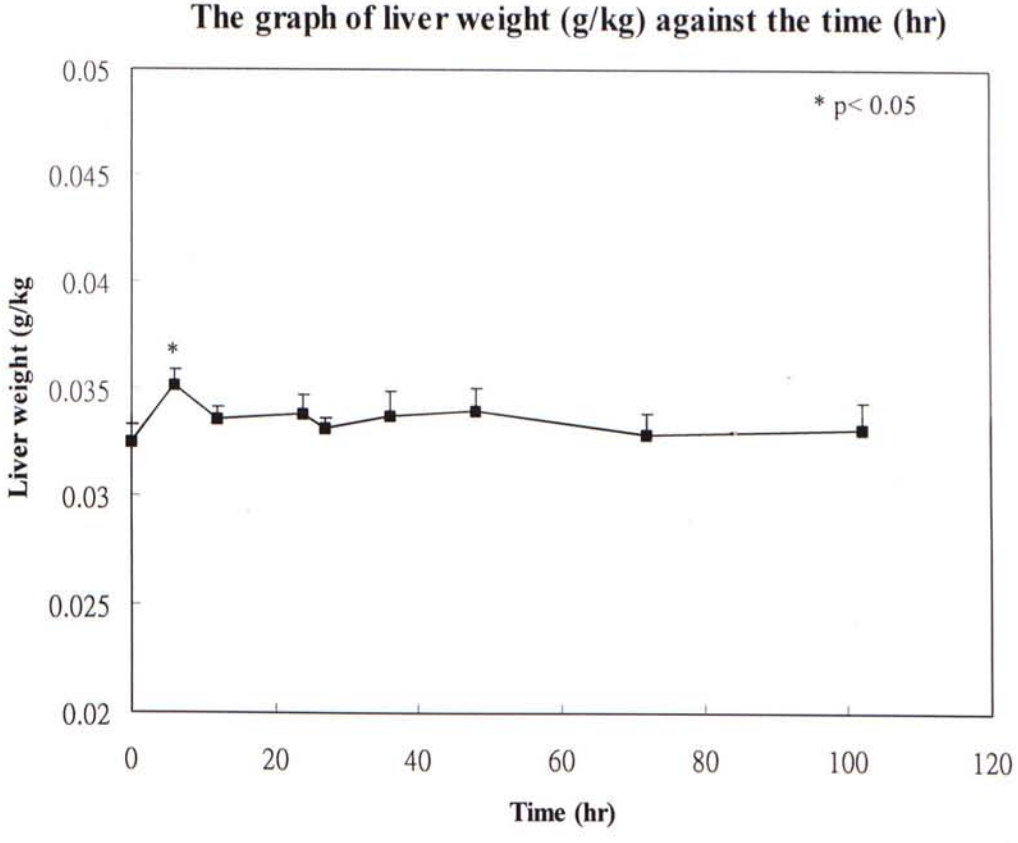


Figure 4.24: Effect on liver weight, i.p. effective dose of 20% TCE in different times (Time course). Each value represents the mean±S.E.M. of corresponding number rats used. Values statistically significantly different from that of vehicle-saline control group (0 h) are shown.

0 h: (N=7); 6 h: (N=8); 12 h: (N=7); 24 h: (N=11); 27 to 102 h: (N=6)

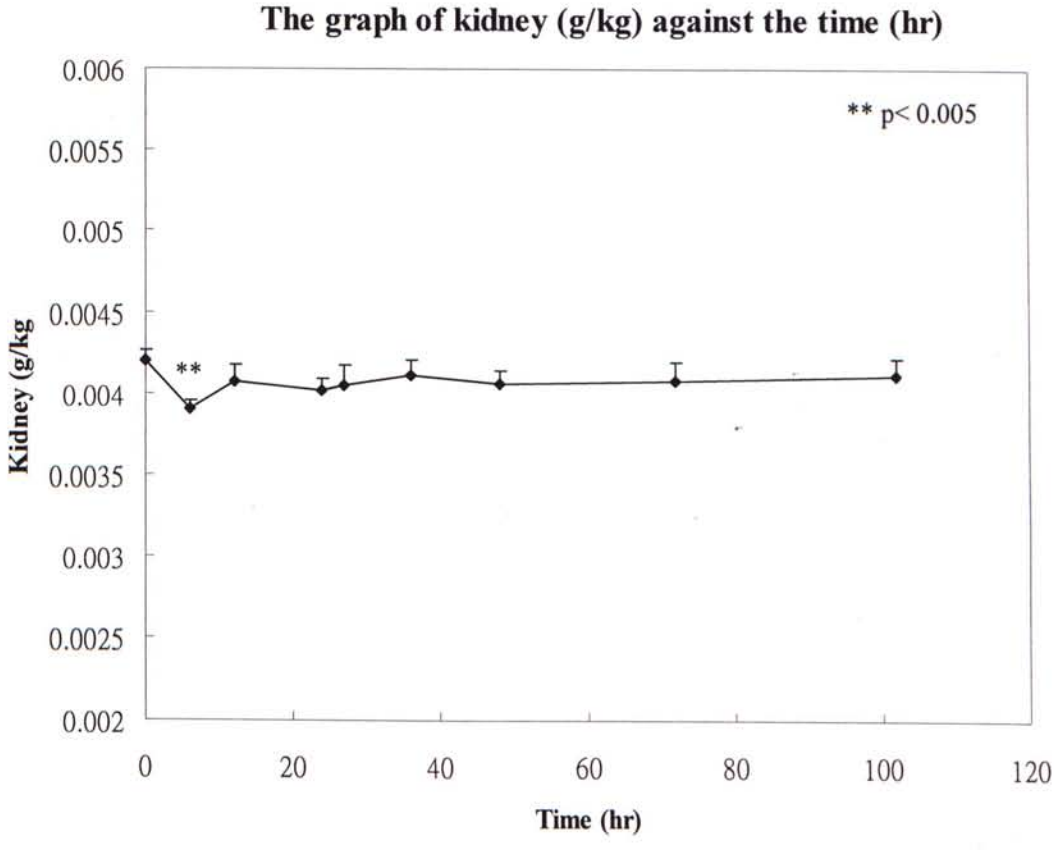


Figure 4.25: Effect on kidney weight, i.p. effective dose of 20% TCE in different times (Time course). Each value represents the mean±S.E.M. of corresponding number of rats used. Values statistically significantly different from that of vehicle-saline control group (0 h) are shown.
0 h: (N=7); 6 h: (N=8); 12 h: (N=7); 24 h: (N=11); 27 to 102 h: (N=6)

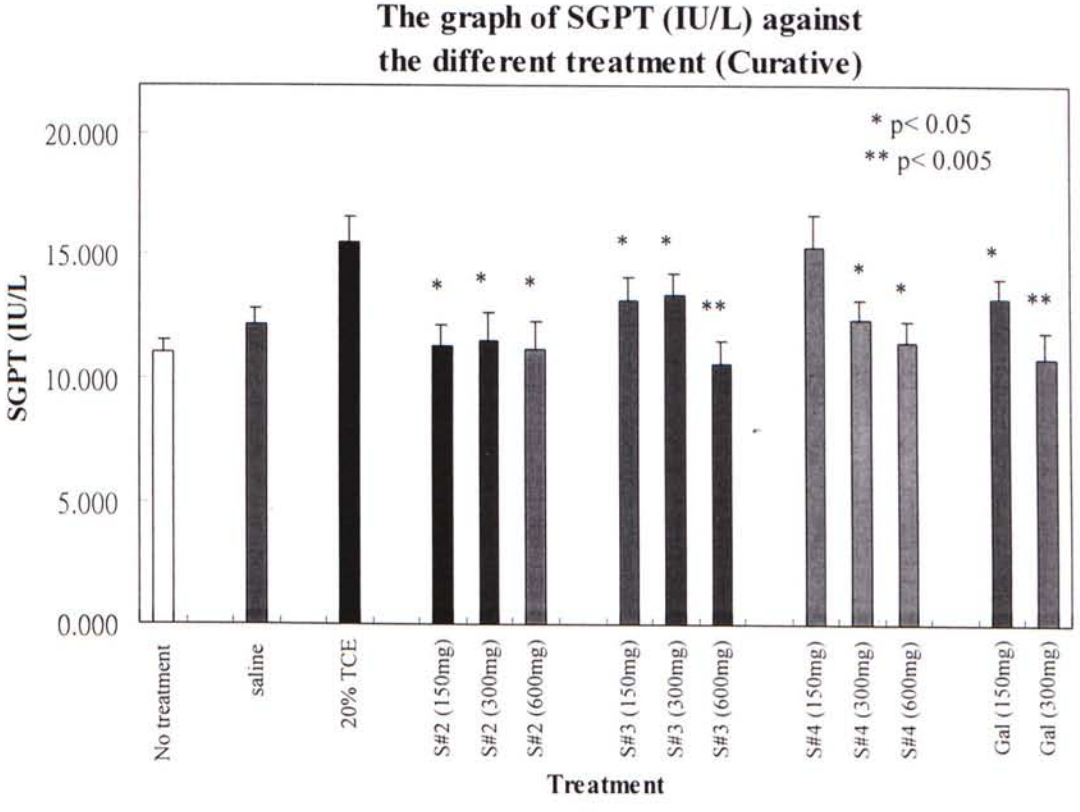


Figure 4.26: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg for brown seaweed; 150 mg/kg and 300 mg/kg for red seaweed) of four species of seaweeds on TCE-induced elevation of SGPT activity (Curative). Each value represents the mean±S.E.M. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

no treatment: (N=5); vehicle-saline: (N=5); 20%TCE: (N=5); S#2, S#3, S#4 and Gal: (N=6)

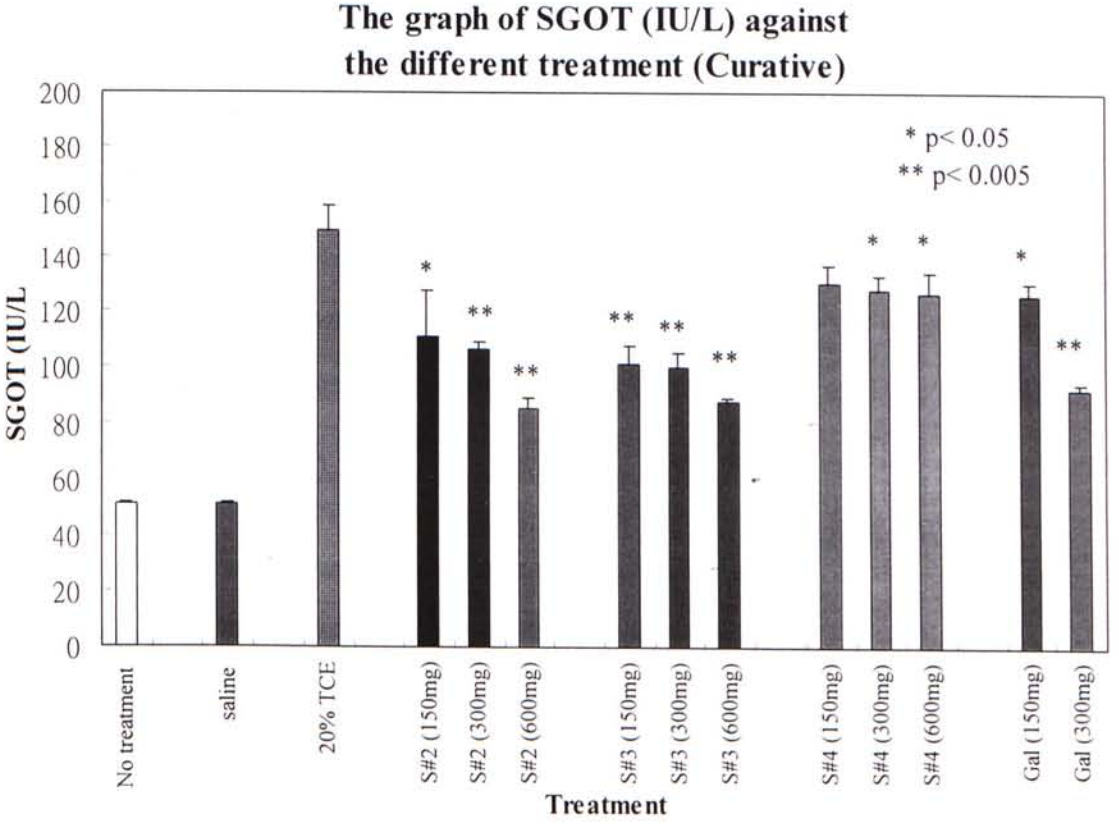


Figure 4.27: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg for brown seaweed; 150 mg/kg and 300 mg/kg for red seaweed) of four species of seaweeds on TCE-induced elevation of SGOT activity (Curative). Each value represents the mean±S.E.M. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

no treatment: (N=5); vehicle-saline: (N=5); 20%TCE: (N=5); S#2, S#3, S#4 and Gal: (N=6)

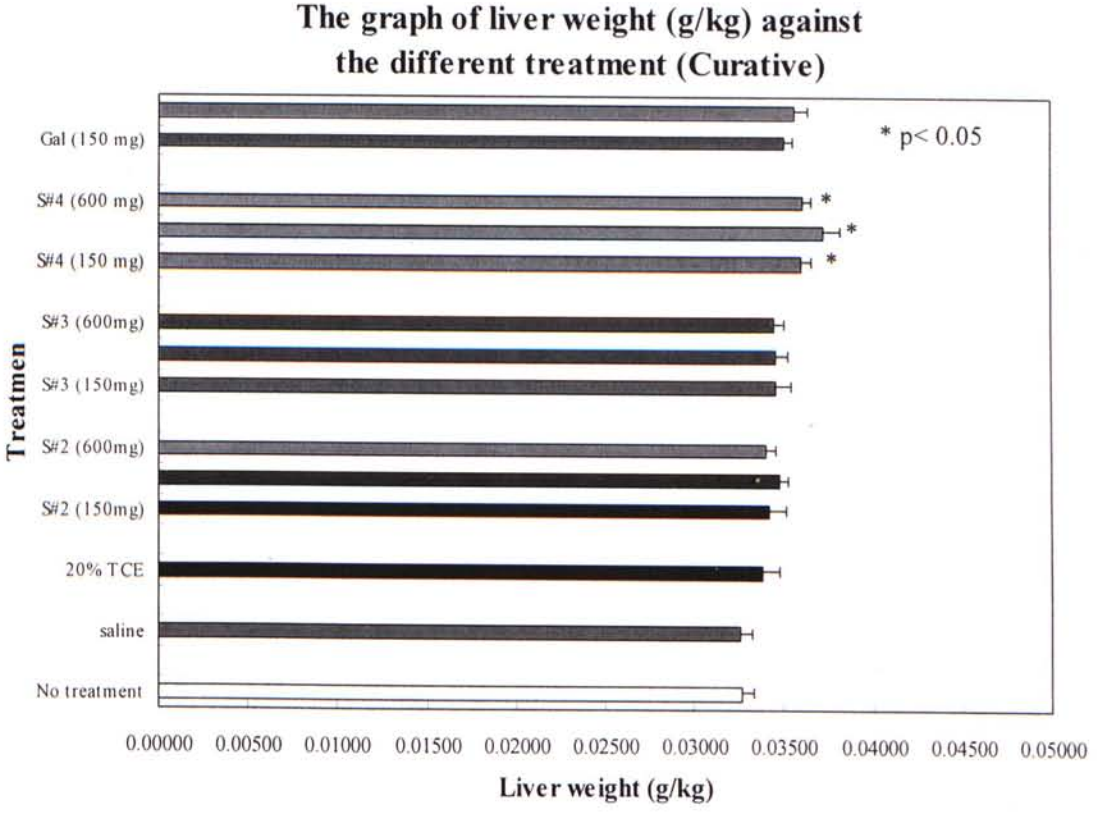


Figure 4.28: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg for brown seaweed; 150 mg/kg and 300 mg/kg for red seaweed) of four species of seaweeds on TCE-treated rats of liver weight (Curative). Each value represents the mean±S.E.M. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and ** ($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

no treatment: (N=5); vehicle-saline: (N=5); 20%TCE: (N=5); S#2, S#3, S#4 and Gal: (N=6)

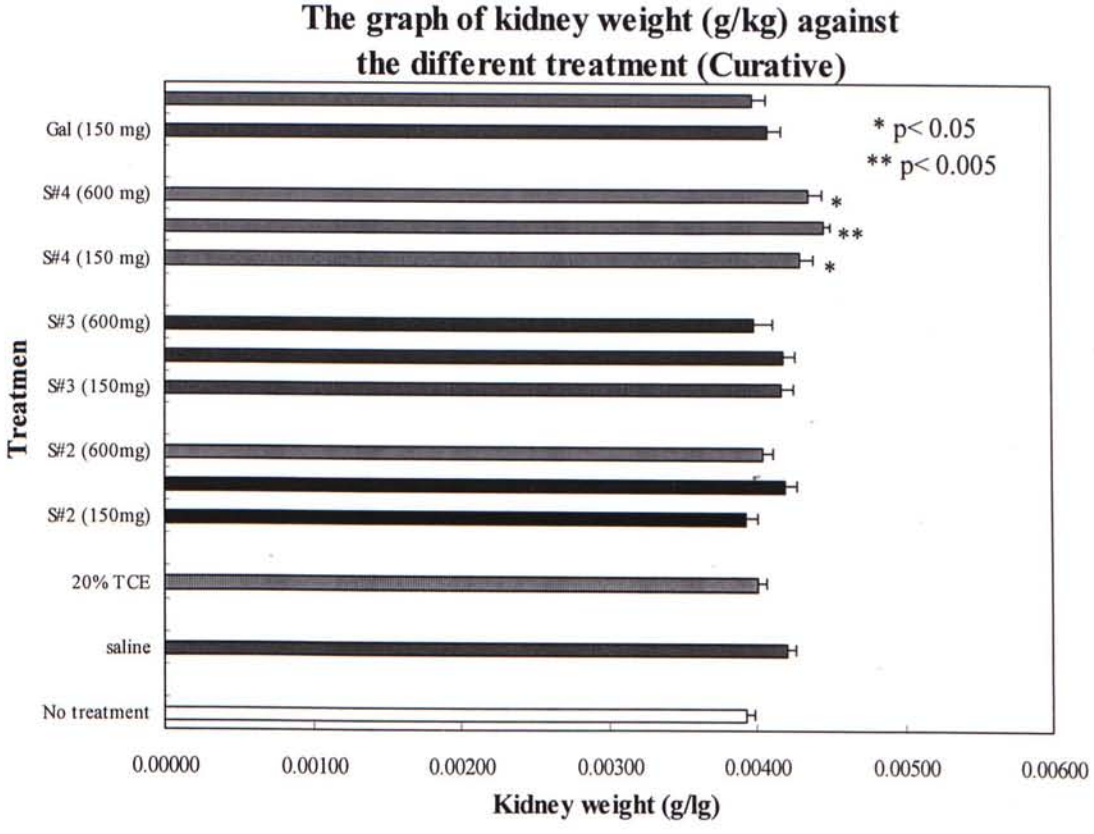


Figure 4.29: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg for brown seaweed; 150 mg/kg and 300 mg/kg for red seaweed) of four species of seaweeds on TCE-treated rats of kidney weight (Curative). Each value represents the mean±S.E.M. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

no treatment: (N=5); vehicle-saline: (N=5); 20%TCE: (N=5); S#2, S#3, S#4 and Gal: (N=6)

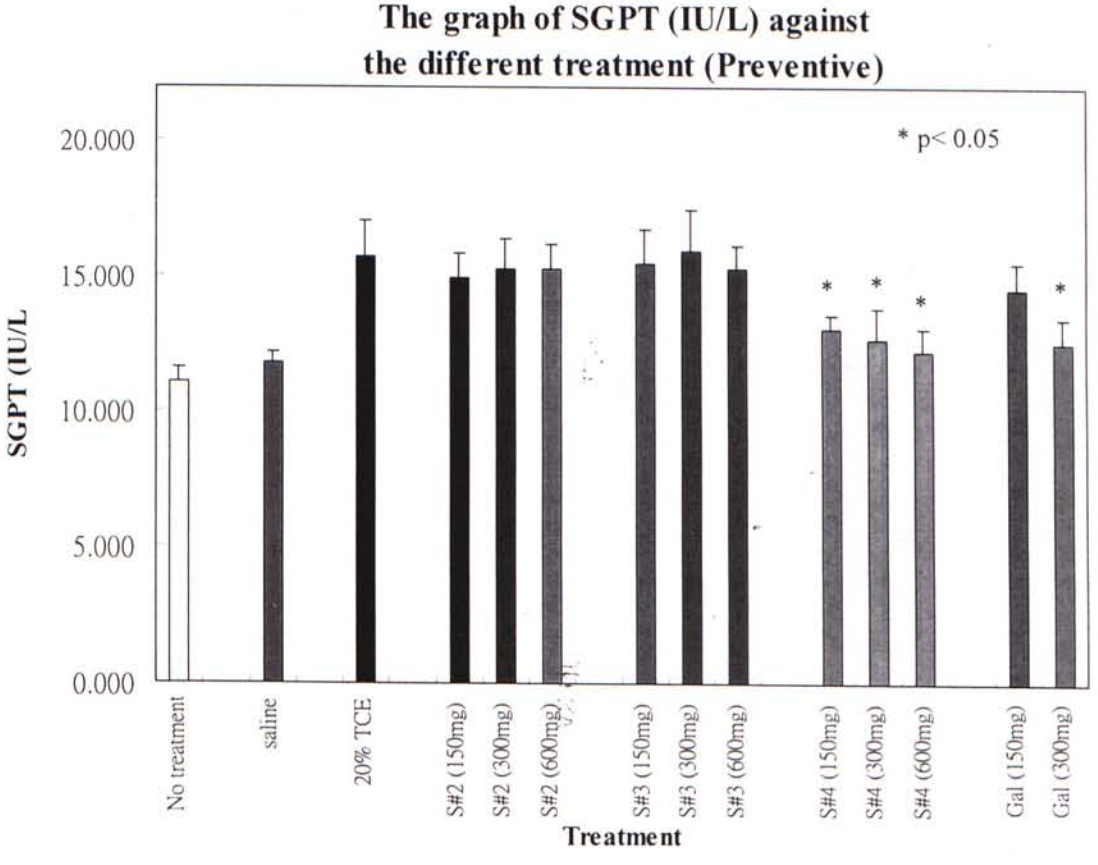


Figure 4.30: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg for brown seaweed; 150 mg/kg and 300 mg/kg for red seaweed) of four species of seaweeds on TCE-induced elevation of SGPT activity (Preventive). Each value represents the mean±S.E.M. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

no treatment: (N=10); vehicle-saline: (N=5); 20%TCE, S#2, S#3, S#4 and Gal: (N=6)

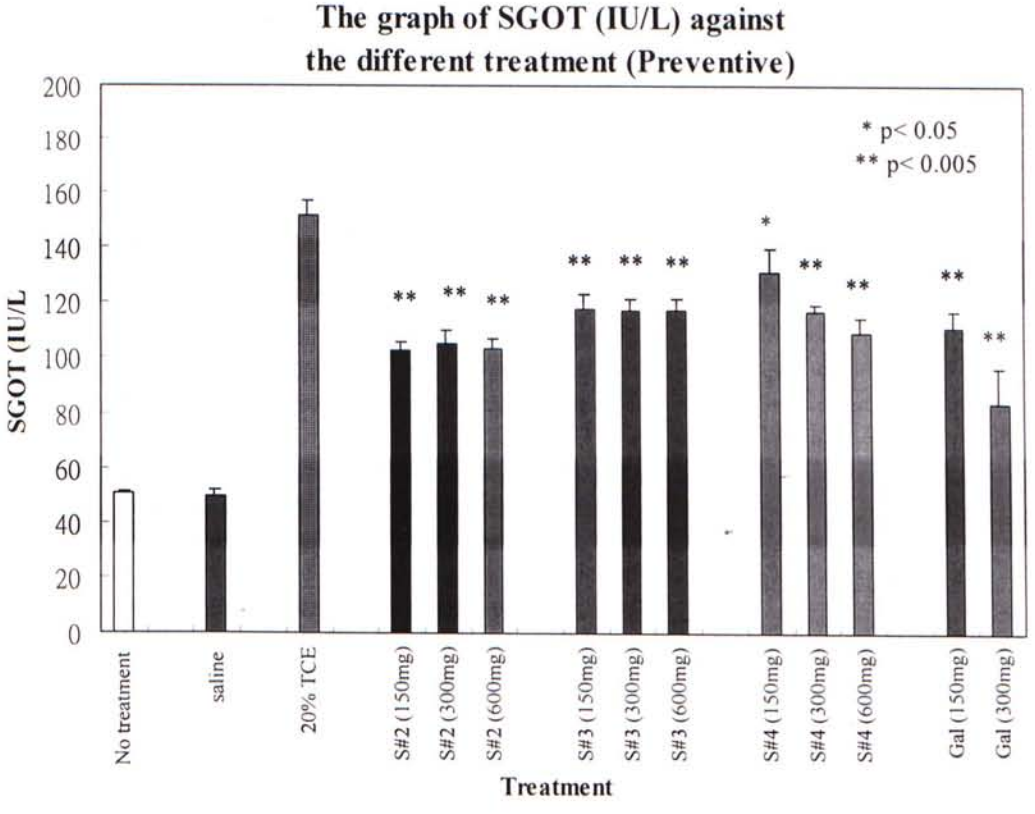


Figure 4.31: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg for brown seaweed; 150 mg/kg and 300 mg/kg for red seaweed) of four species of seaweeds on TCE-induced elevation of SGOT activity (Preventive). Each value represents the mean±S.E.M. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$), ** ($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

no treatment: (N=10); vehicle-saline: (N=5); 20%TCE, S#2, S#3, S#4 and Gal: (N=6)

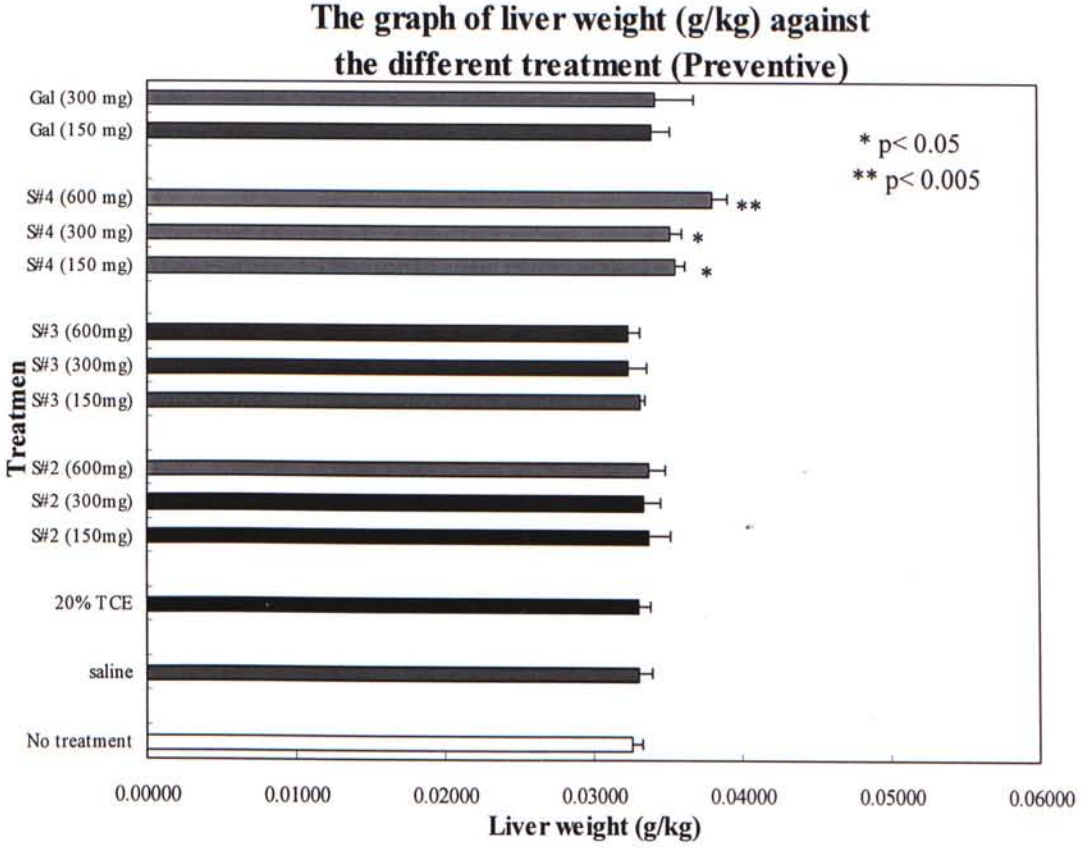


Figure 4.32: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg for brown seaweed; 150 mg/kg and 300 mg/kg for red seaweed) of four species of seaweeds on liver weight of TCE-treated rats (Preventive). Each value represents the mean±S.E.M. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

no treatment: (N=10); vehicle-saline: (N=5); 20%TCE, S#2, S#3, S#4 and Gal: (N=6)

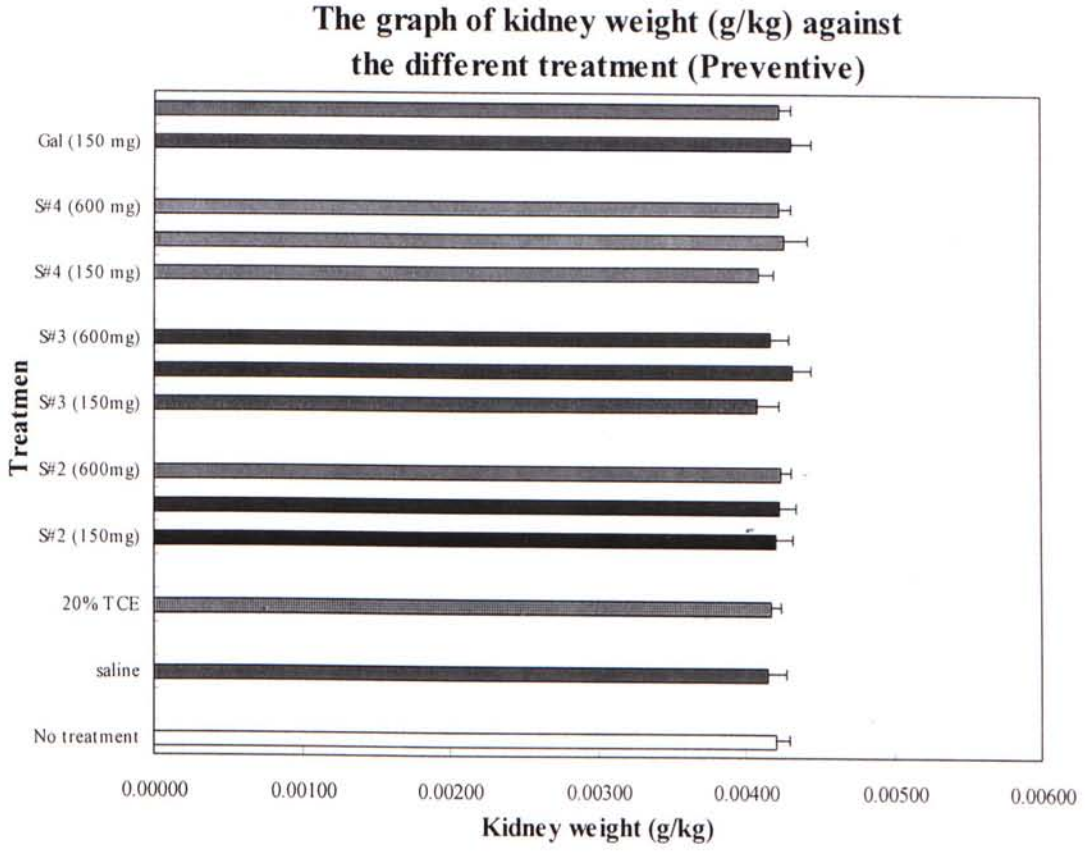


Figure 4.33: Effect of extract (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/ kg for brown seaweed; 150 mg/kg and 300 mg/kg for red seaweed) of four species of seaweeds on kidney weight of TCE-treated rats (Preventive). Each value represents the mean±S.E.M. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

no treatment: (N=10); vehicle-saline: (N=5); 20%TCE, S#2, S#3, S#4 and Gal: (N=6)

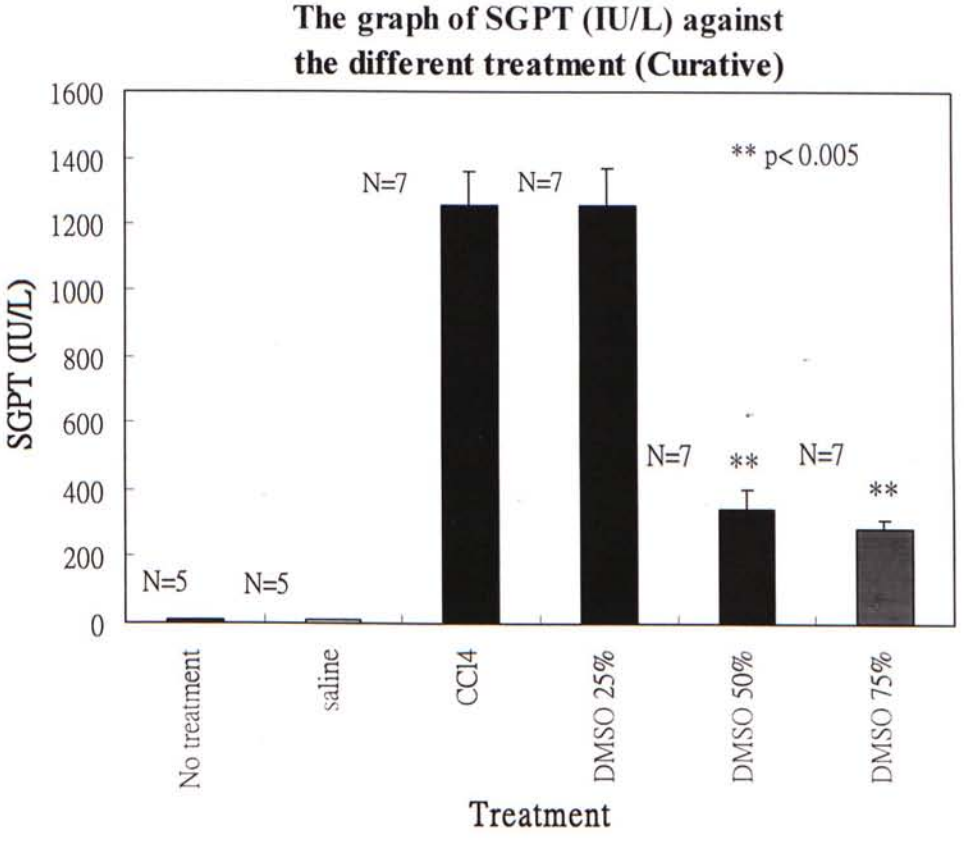


Figure 4.34: Effect of DMSO (at dosages of 25%, 50% and 75%) on CCl_4-induced elevation of SGPT activity (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

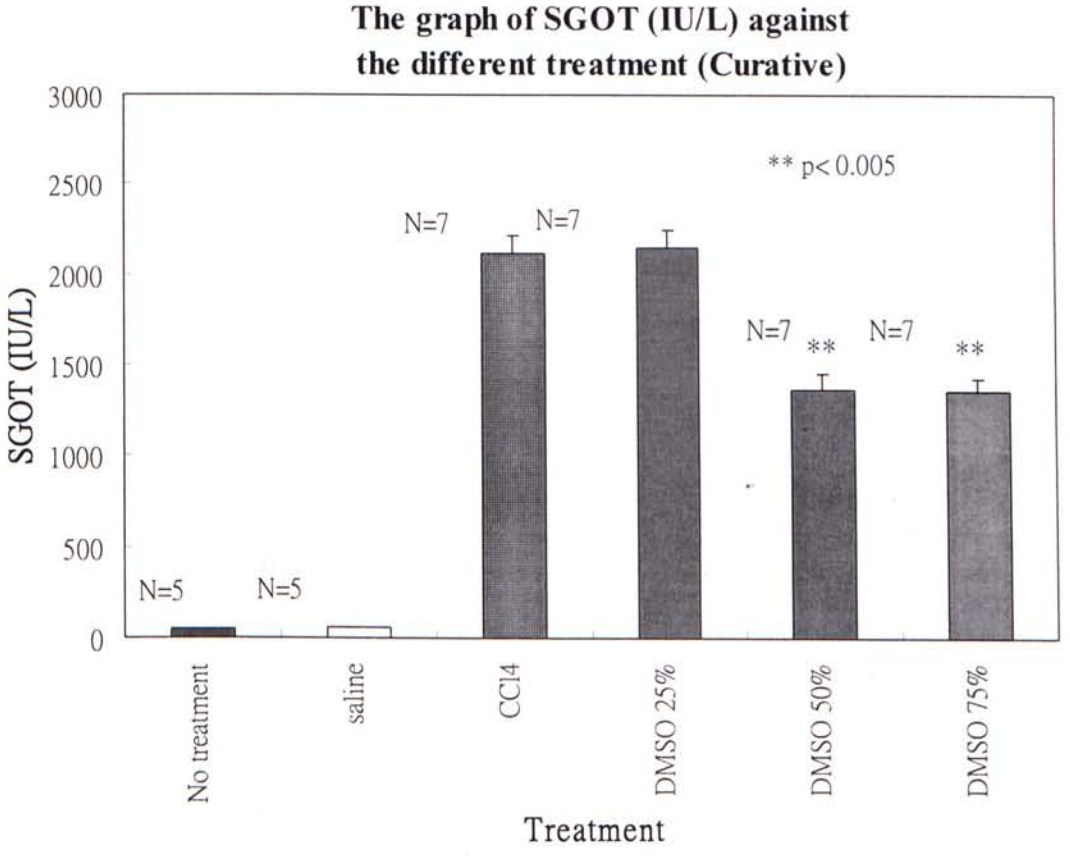


Figure 4.35: Effect of DMSO (at dosages of 25%, 50% and 75%) on CCl_4-induced elevation of SGOT activity (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

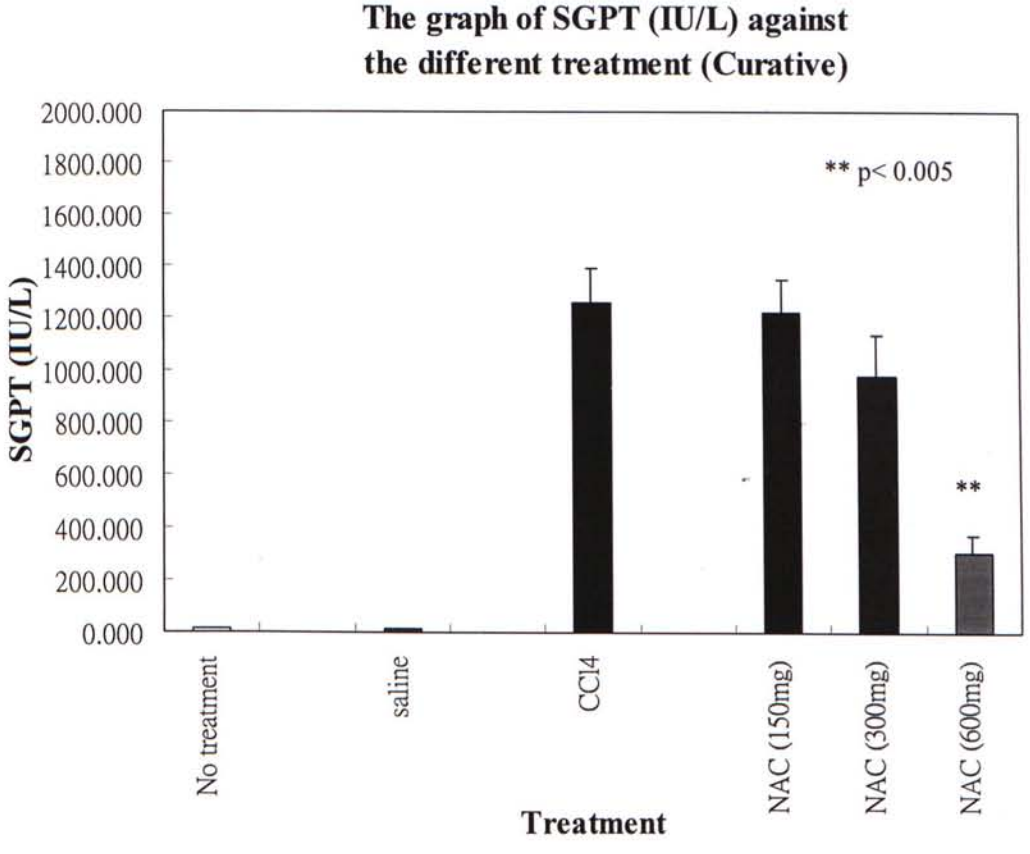


Figure 4.36: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on CCl_4-induced elevation of SGPT activity (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by **($p<0.005$).

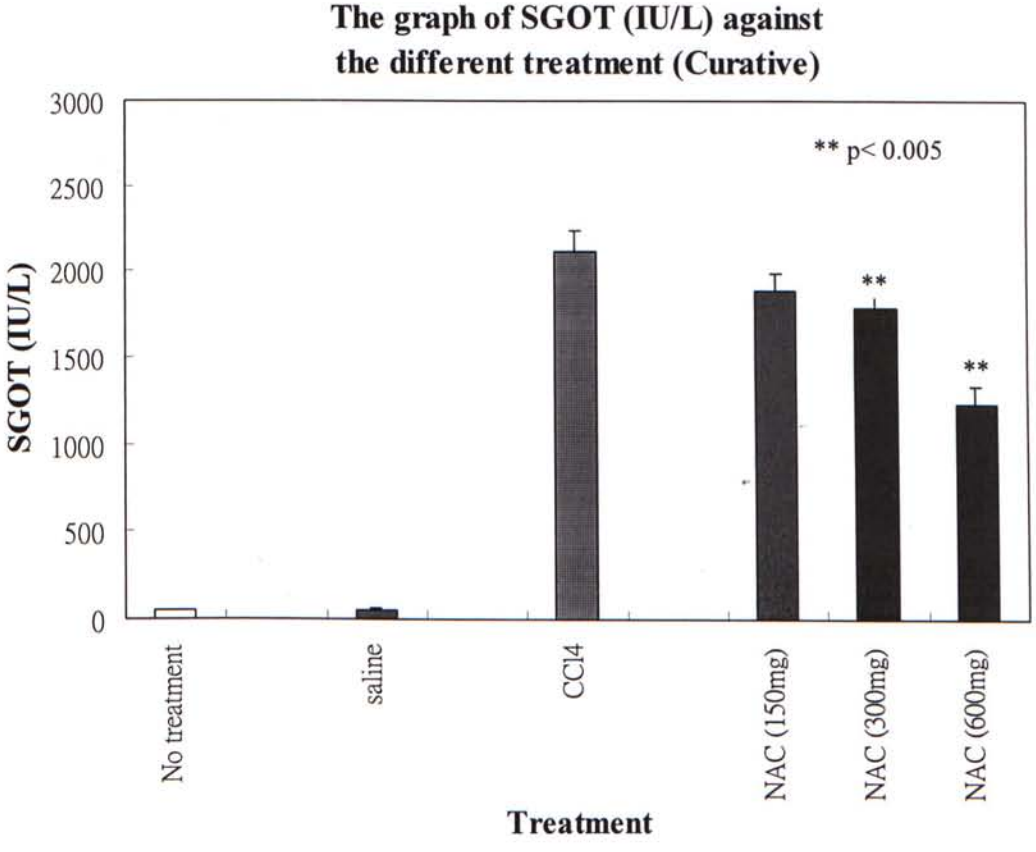


Figure 4.37: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on CCl_4-induced elevation of SGOT activity (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by **($p<0.005$).

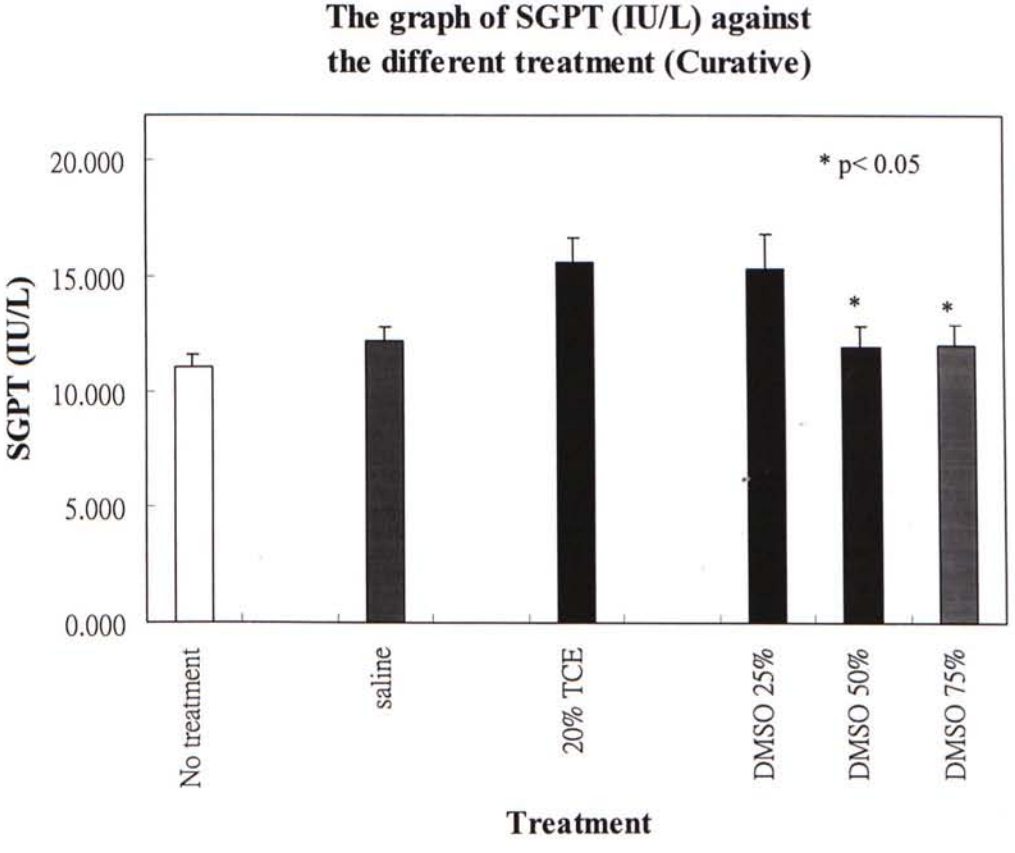


Figure 4.38: Effect of DMSO (at dosages of 25%, 50% and 75%) on TCE-induced elevation of SGPT activity (Curative). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and ** ($p<0.005$).

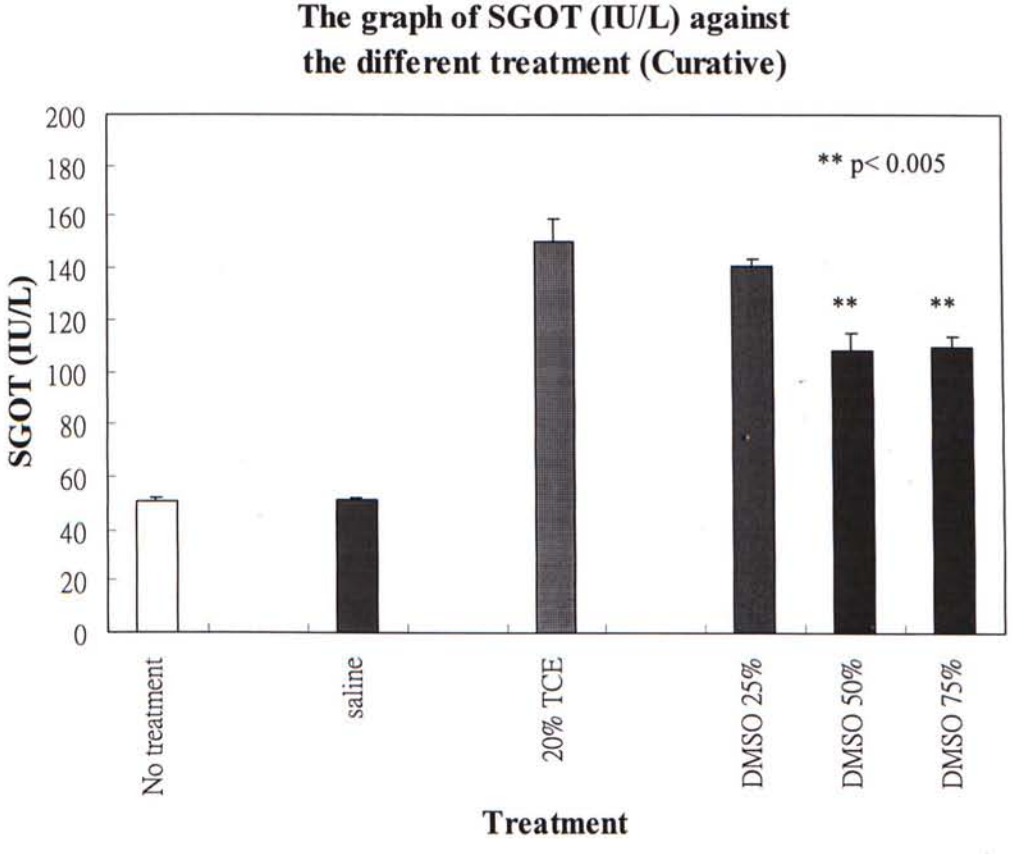


Figure 4.39: Effect of DMSO (at dosages of 25%, 50% and 75%) on TCE-induced elevation of SGOT activity (Curative). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and ** ($p<0.005$).

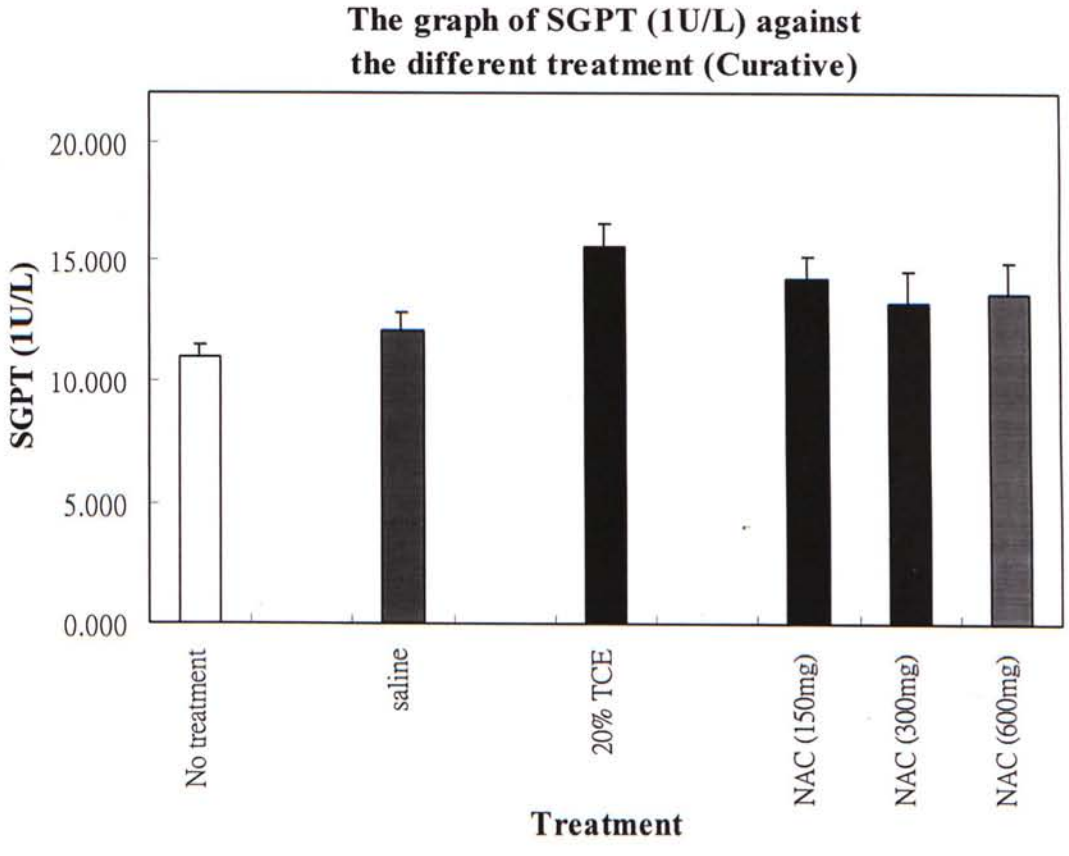


Figure 4.40: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on TCE-induced elevation of SGPT activity (Curative). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group is not noted.

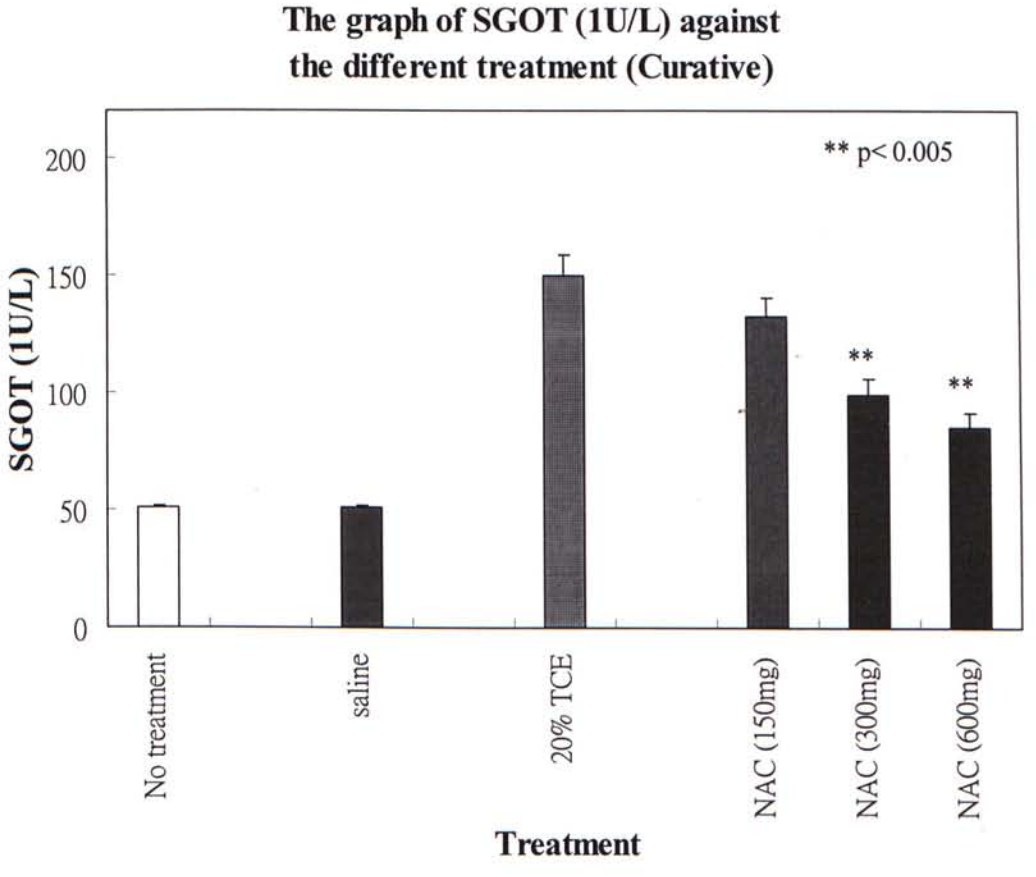


Figure 4.41: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on TCE-induced elevation of SGOT activity (Curative). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by **($p<0.005$).

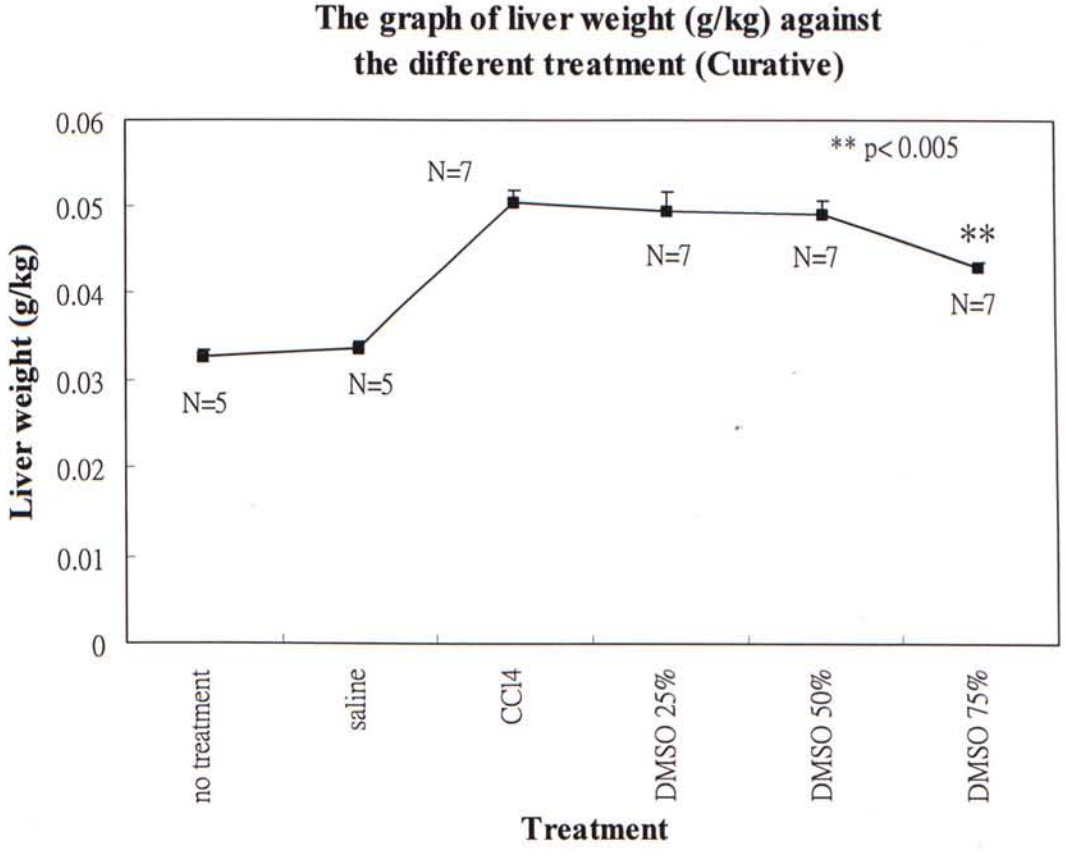


Figure 4.42: Effect of DMSO (at dosages of 25%, 50% and 75%) on CCl_4-induced increase of liver weight (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

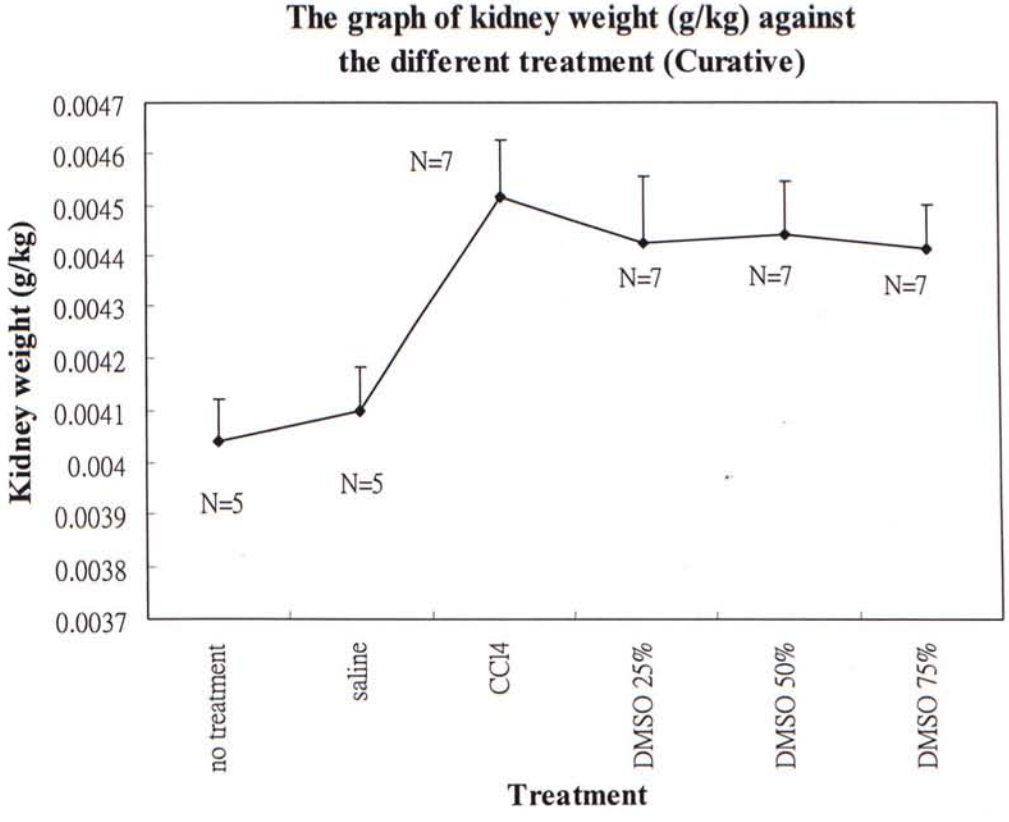


Figure 4.43: Effect of DMSO (at dosages of 25%, 50% and 75%) on CCl_4-induced increase of kidney weight (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

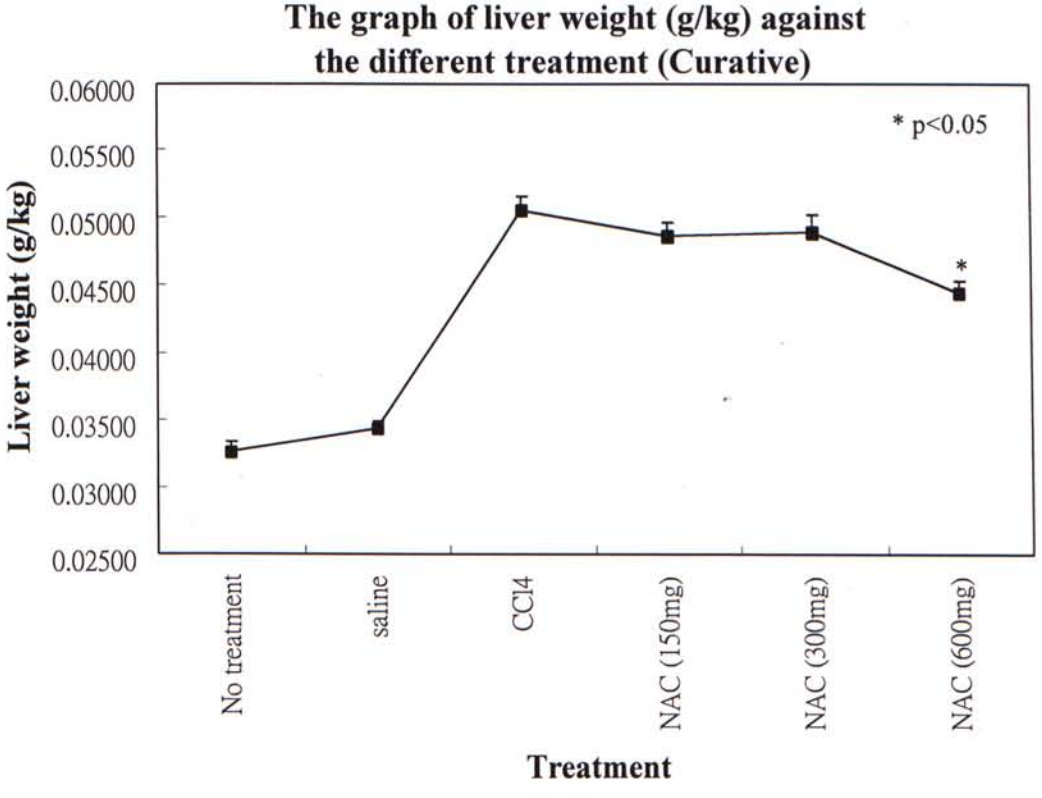


Figure 4.44: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on CCl_4-induced increase of liver weight (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

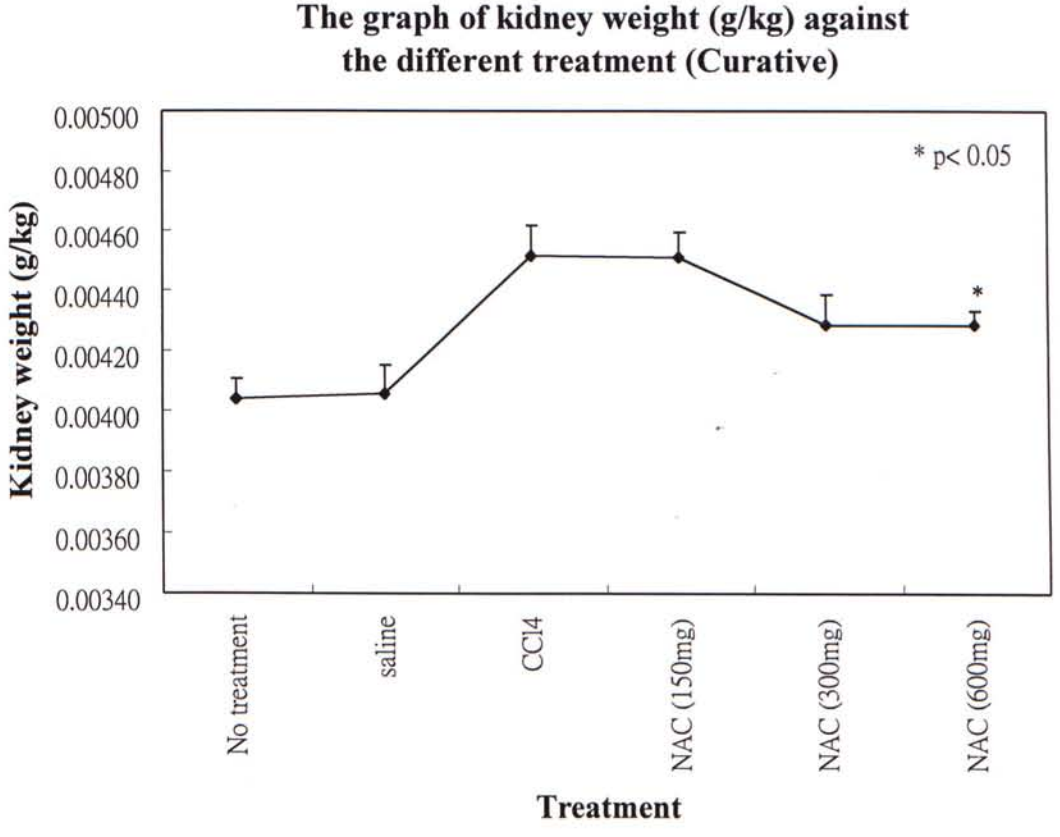


Figure 4.45: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on CCl_4-induced increase of kidney weight (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$).

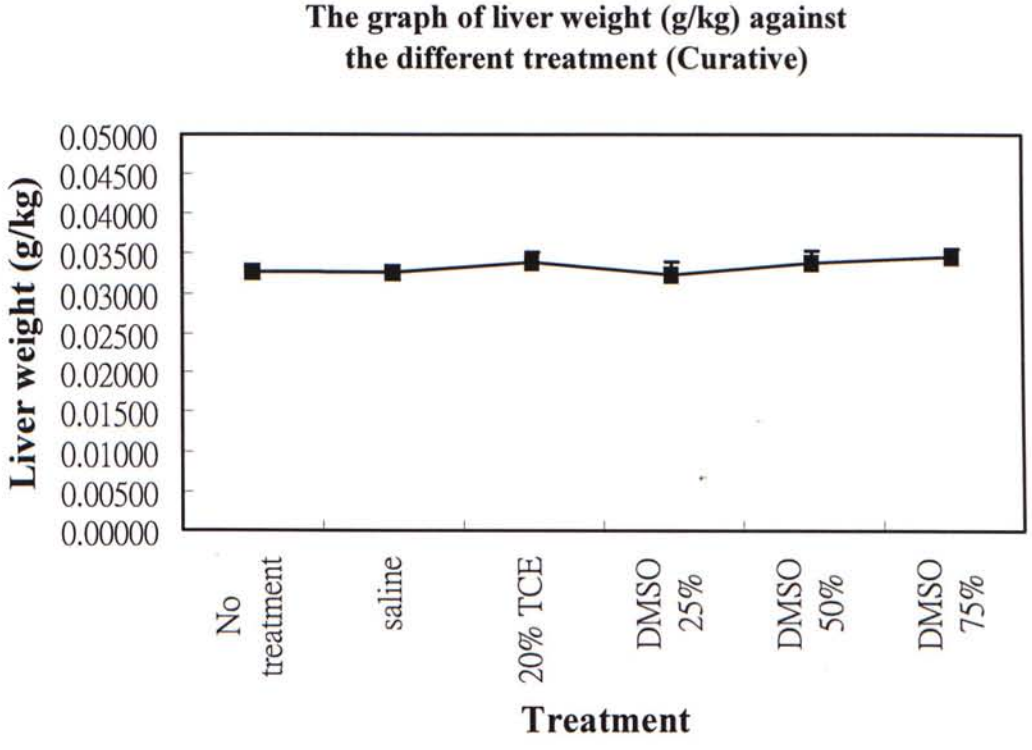


Figure 4.46: Effect of DMSO (at dosages of 25%, 50% and 75%) on liver weight of TCE-treated rats (Curative). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

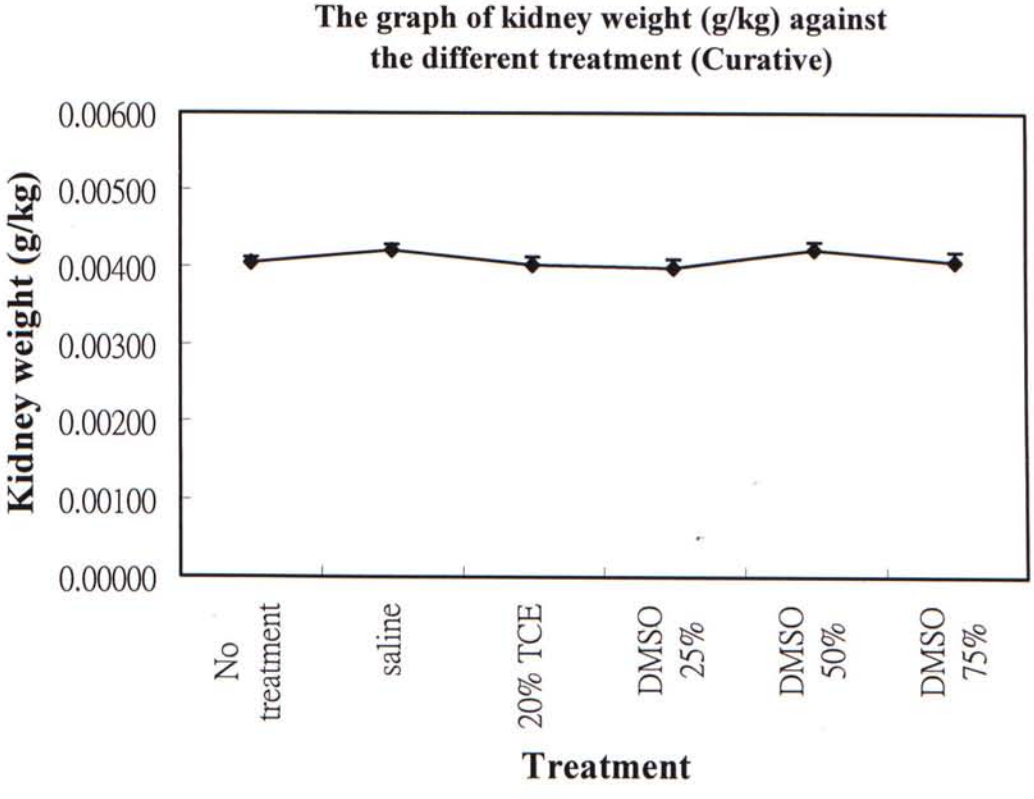


Figure 4.47: Effect of DMSO (at dosages of 25%, 50% and 75%) on kidney weight of TCE-treated rats (Curative). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

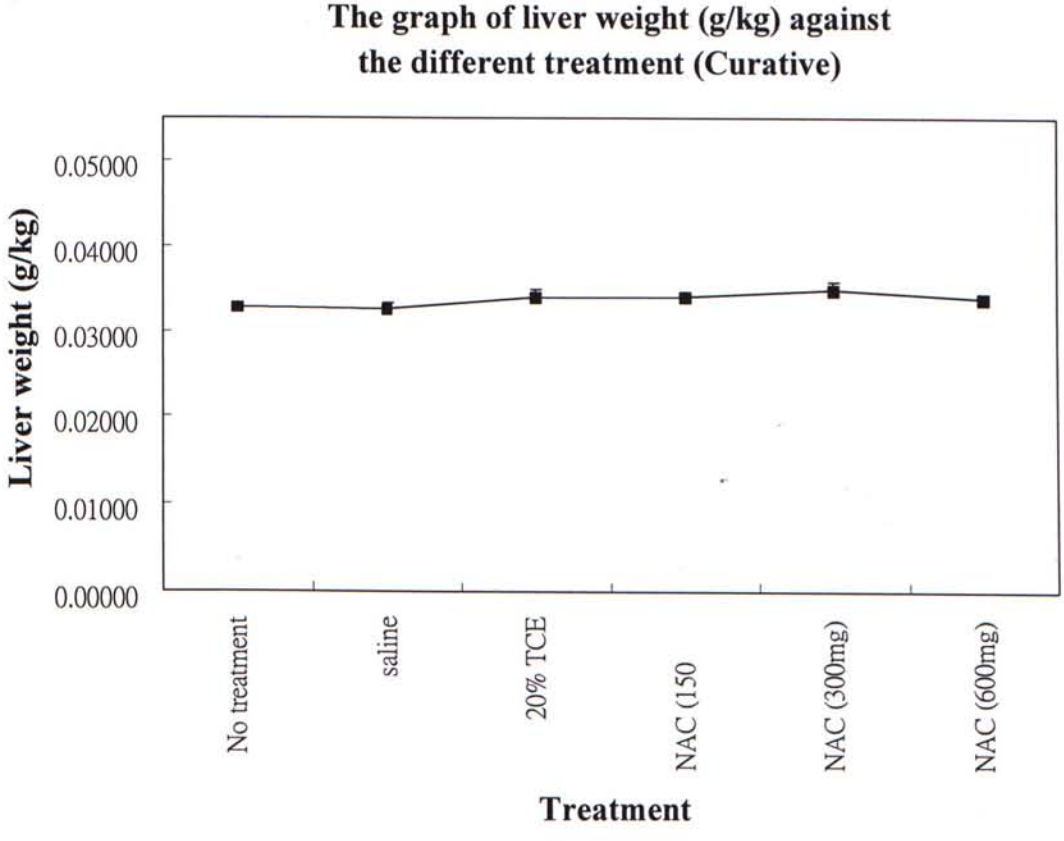


Figure 4.48: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on liver weight of TCE-treated rats (Curative). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

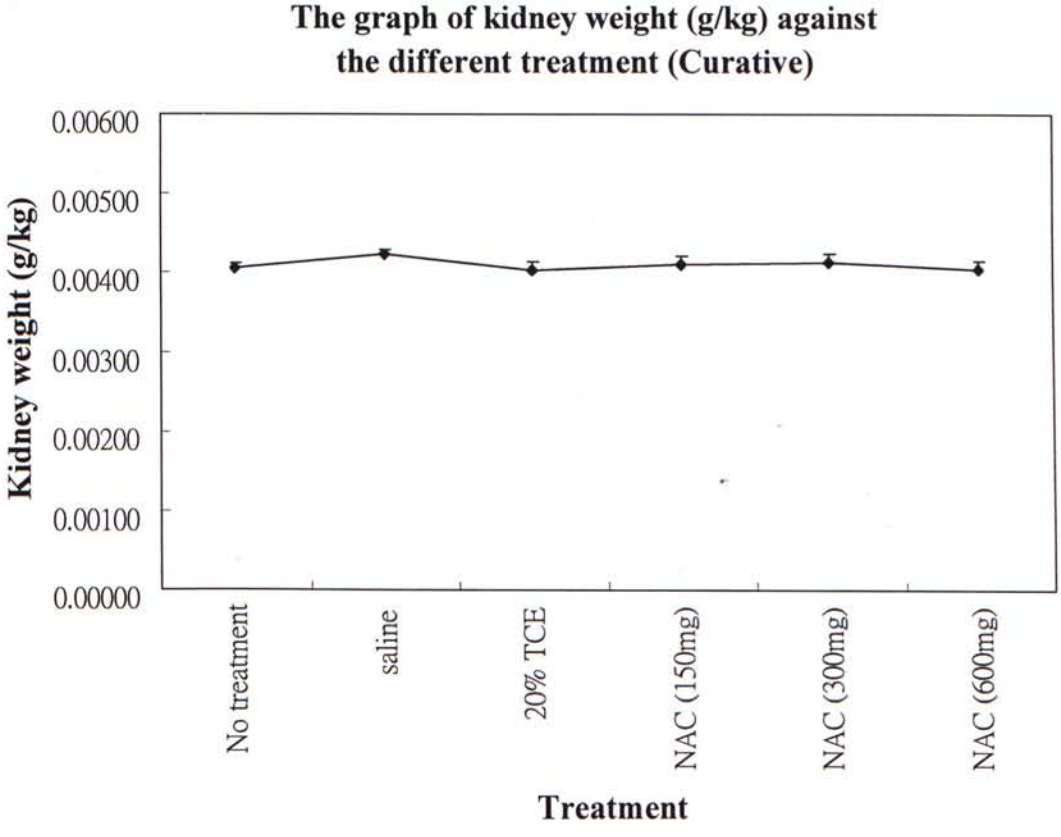


Figure 4.49: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on kidney weight of TCE-treated rats (Curative). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

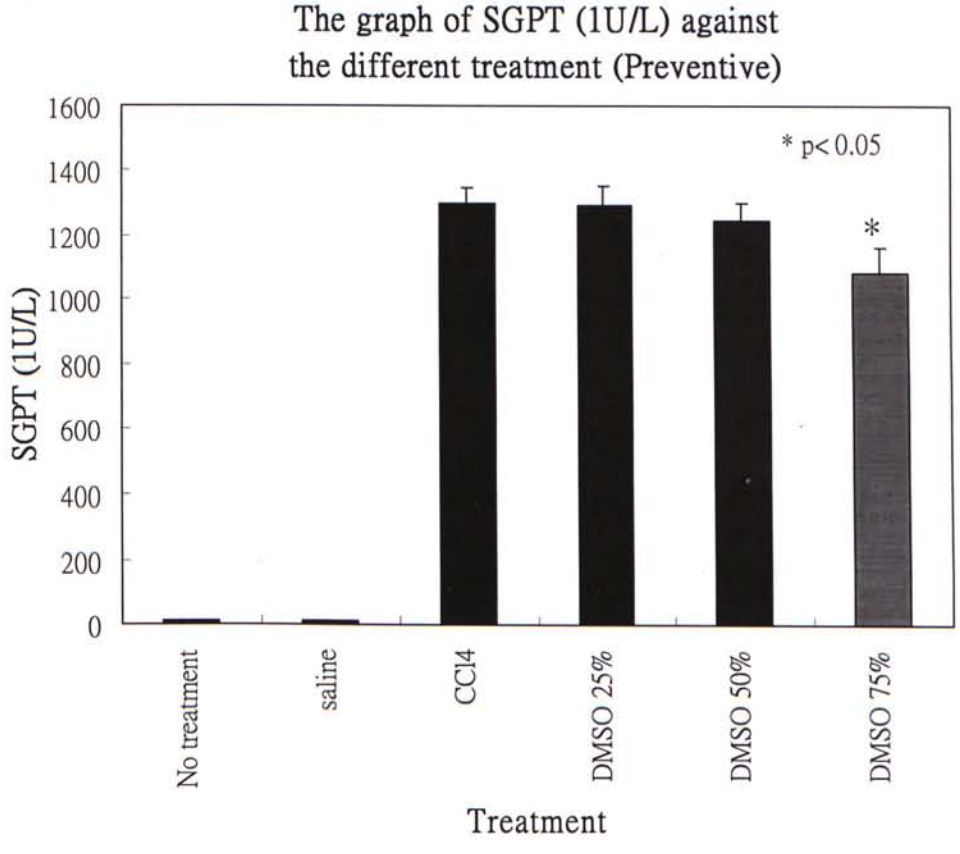


Figure 4.50: Effect of DMSO (at dosages of 25%, 50% and 75%) on CCl_4-induced elevation of SGPT activity (Preventive). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$).

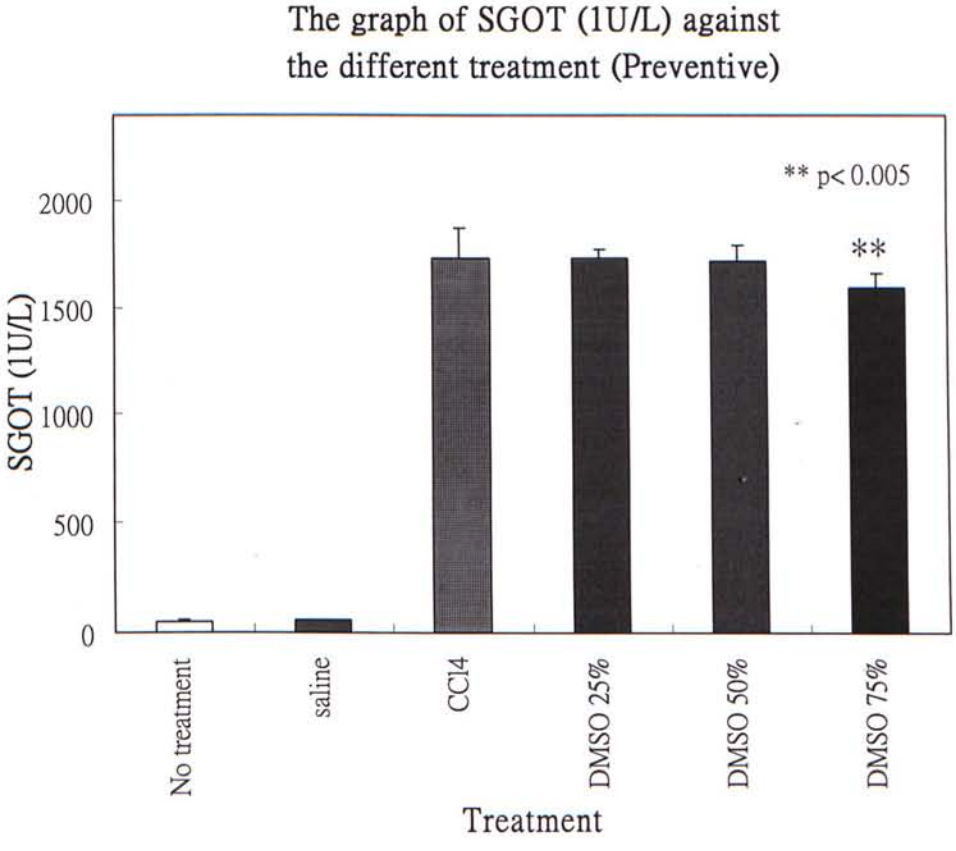


Figure 4.51: Effect of DMSO (at dosages of 25%, 50% and 75%) on CCl_4-induced elevation of SGOT activity (Preventive). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by **($p<0.005$).

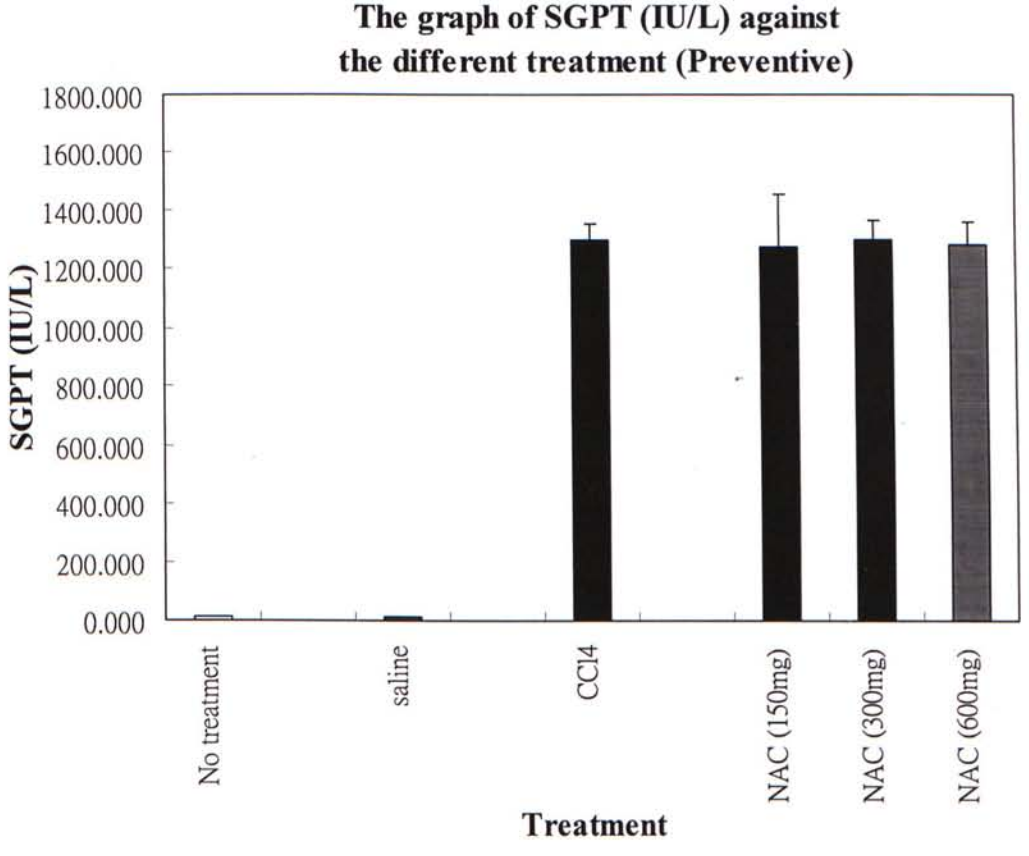


Figure 4.52: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on CCl_4-induced elevation of SGPT activity (Preventive). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

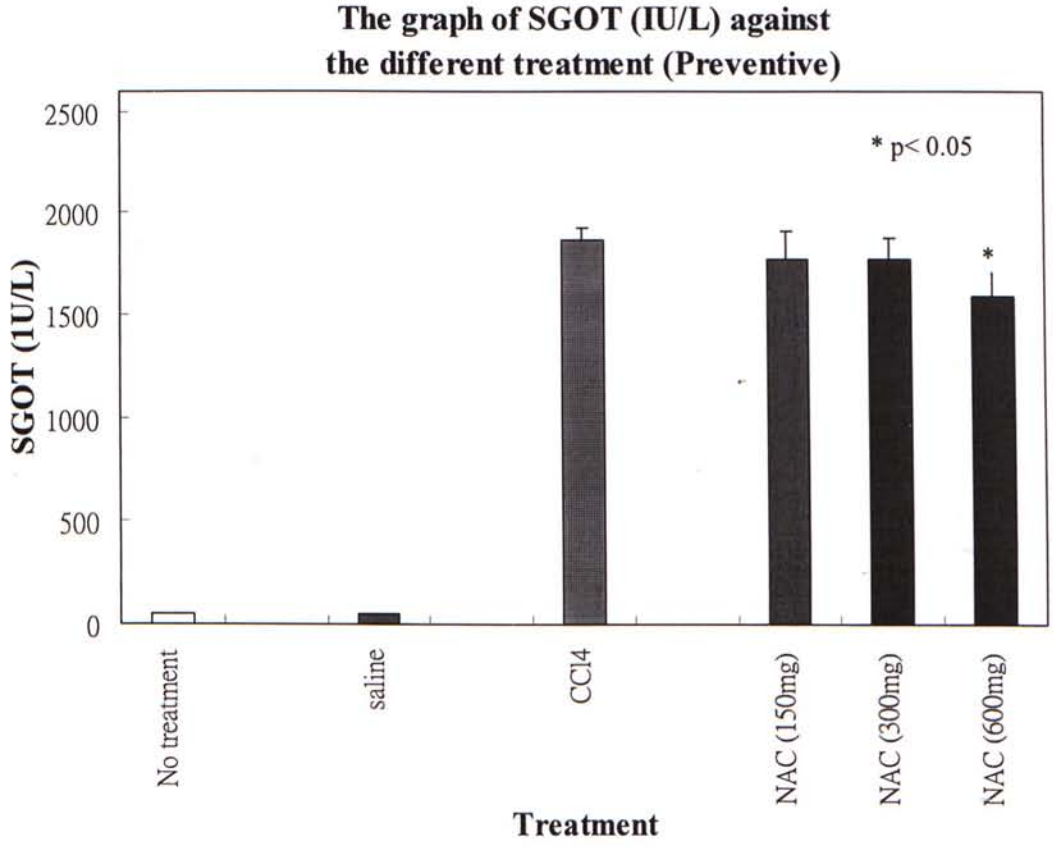


Figure 4.53: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on CCl_4-induced elevation of SGOT activity (Preventive). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by *($p<0.05$).

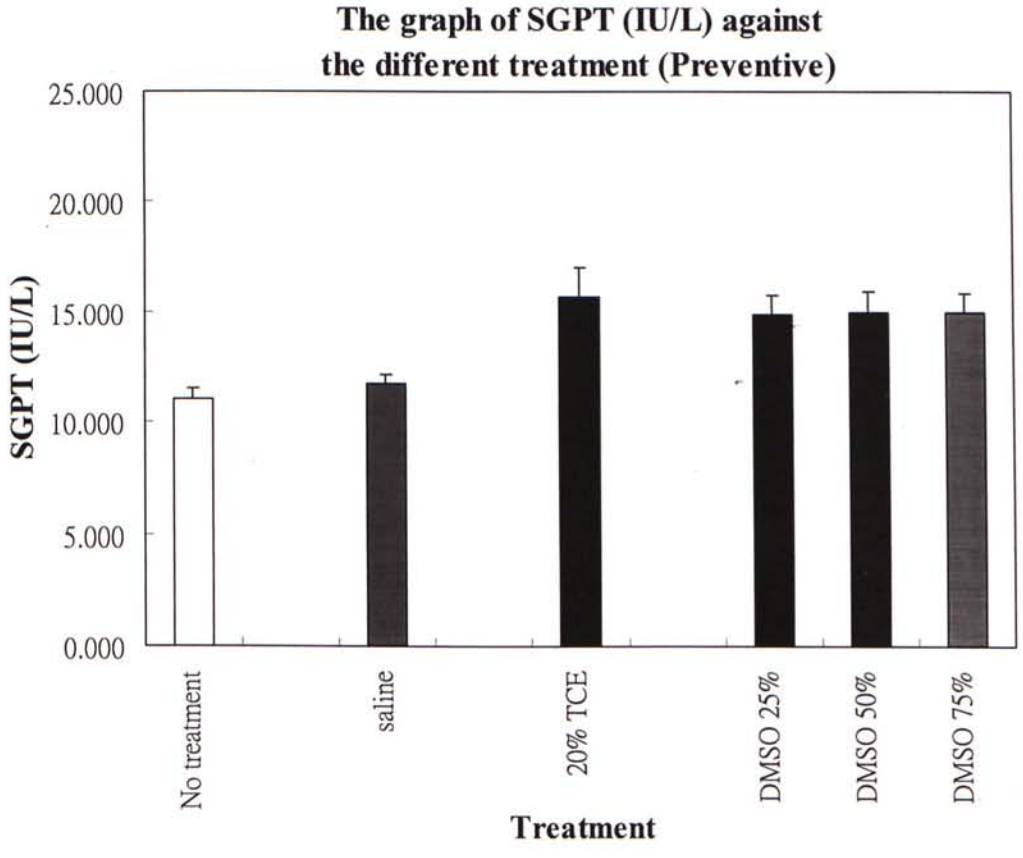


Figure 4.54: Effect of DMSO (at dosages of 25%, 50% and 75%) on TCE-induced elevation of SGPT activity (Preventive). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

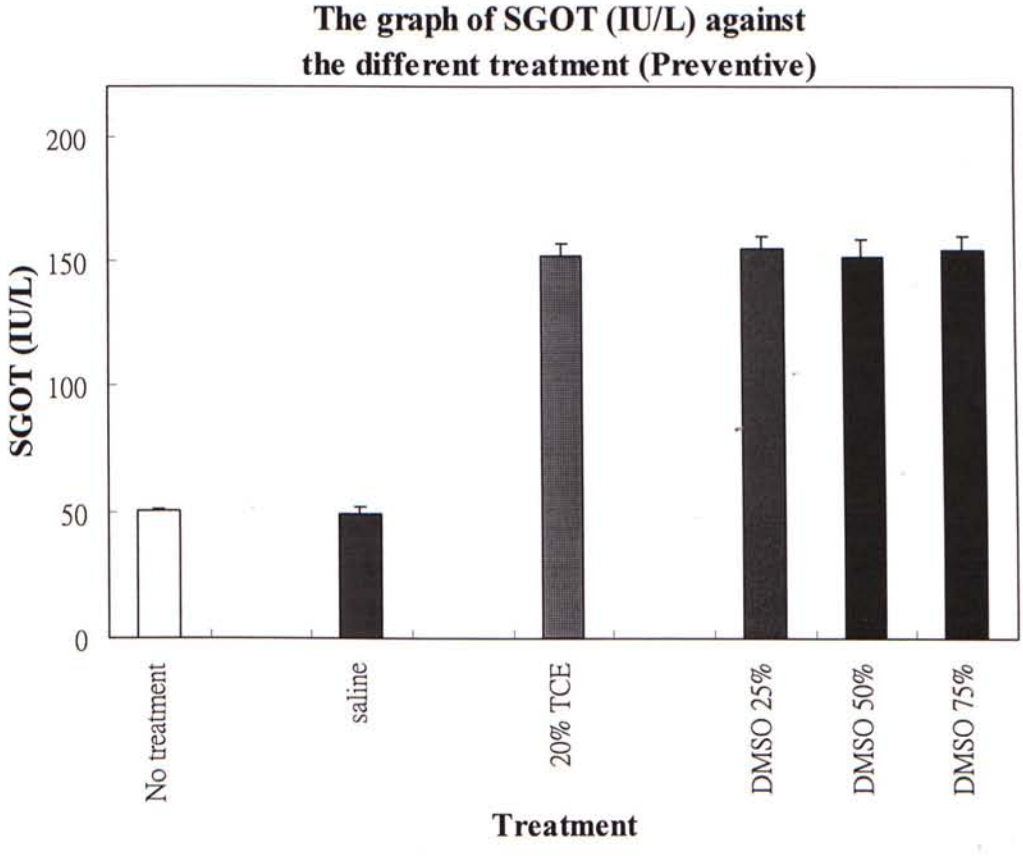


Figure 4.55: Effect of DMSO (at dosages of 25%, 50% and 75%) on TCE-induced elevation of SGOT activity (Preventive). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

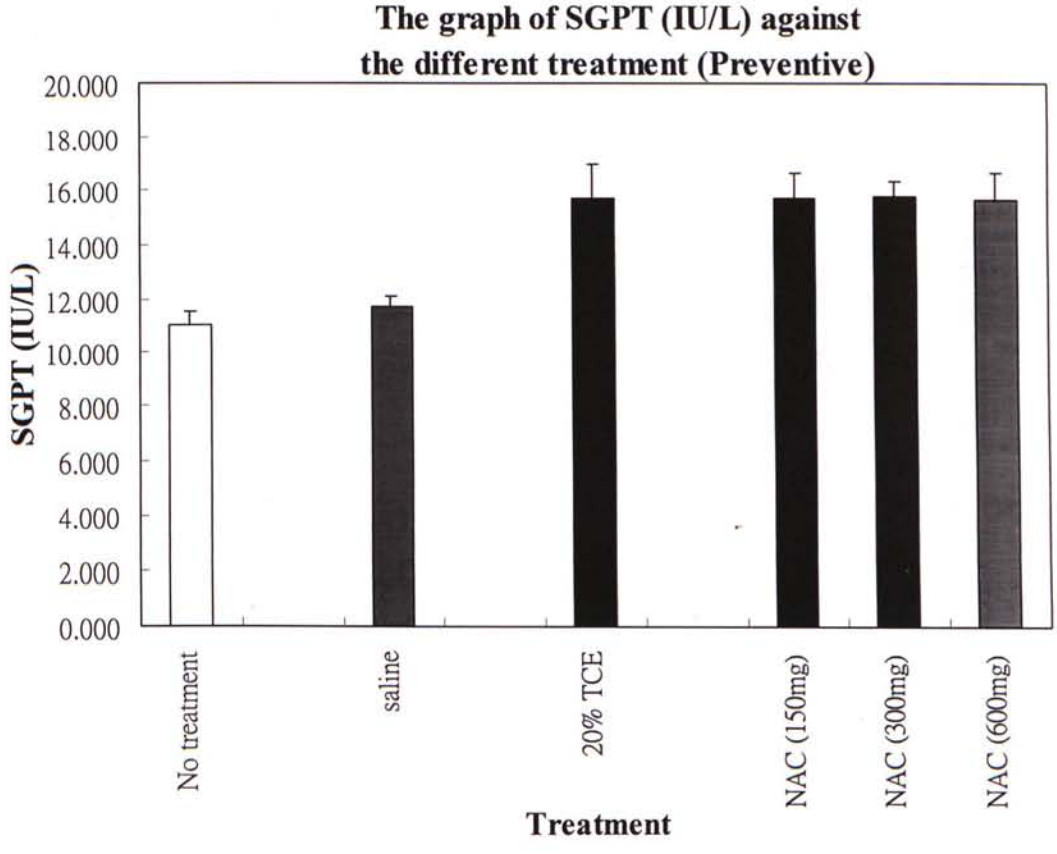


Figure 4.56: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on TCE-induced elevation of SGPT activity (Preventive). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group is not noted.

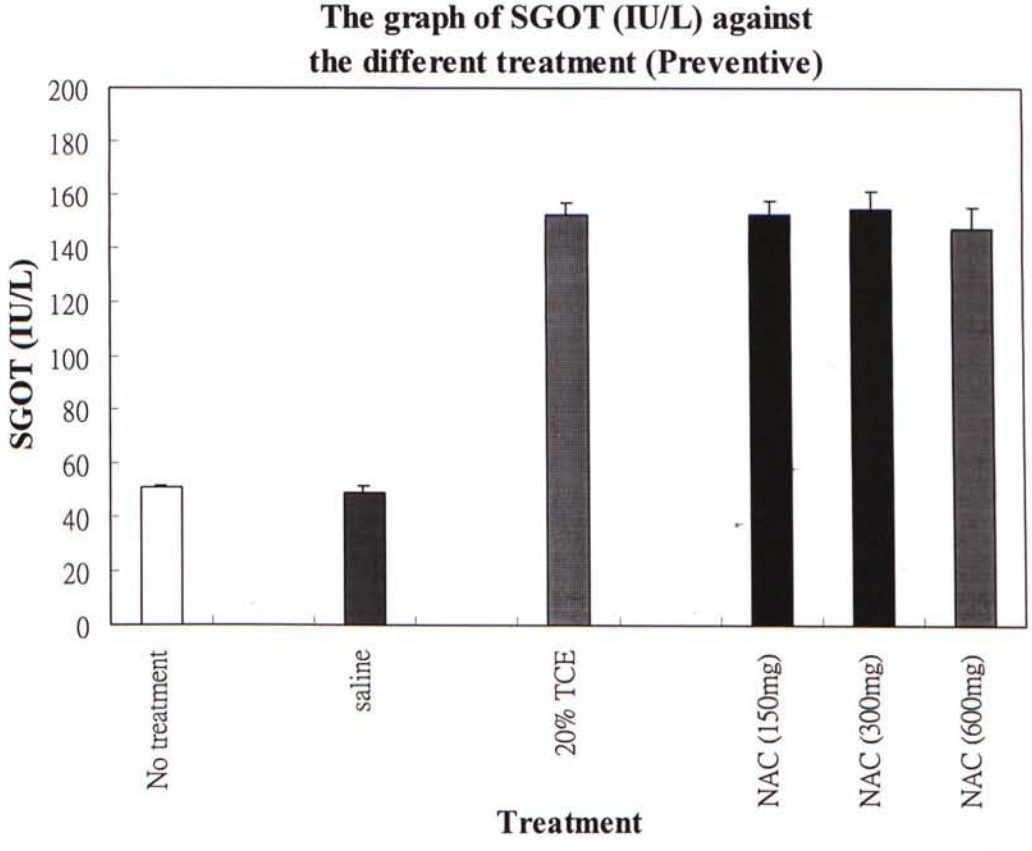


Figure 4.57: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on TCE-induced elevation of SGOT activity (Preventive). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

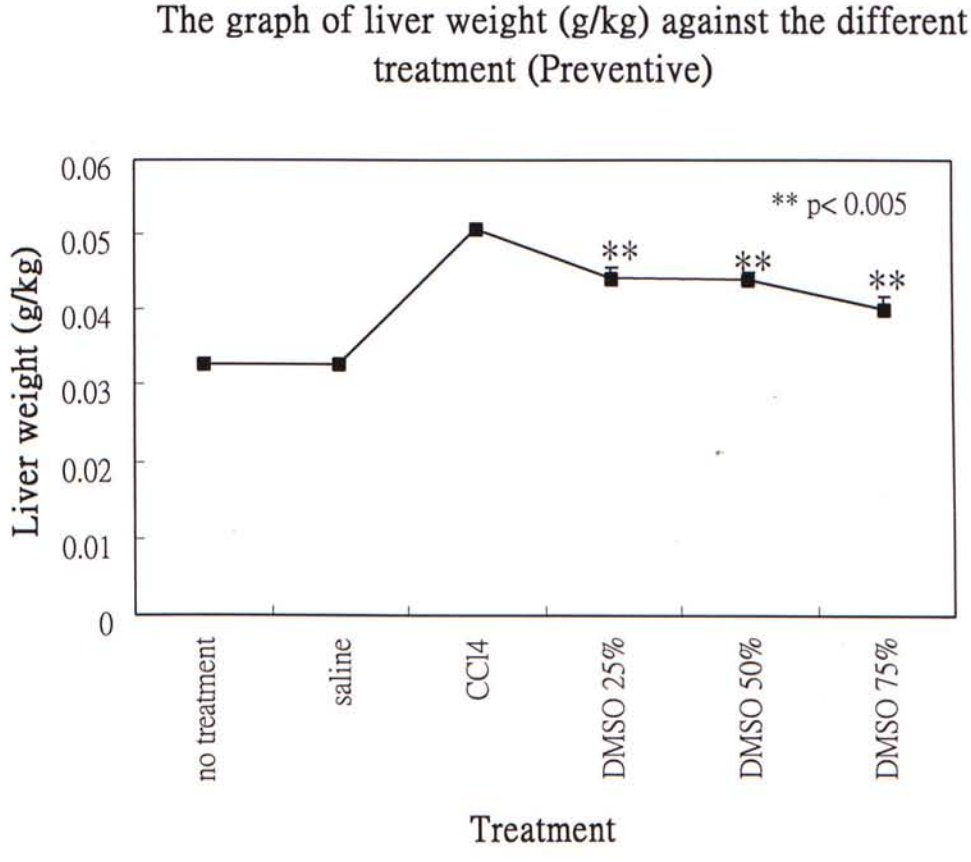


Figure 4.58: Effect of DMSO (at dosages of 25%, 50% and 75%) on CCl_4-induced increase of liver weight (Preventive). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by **($p<0.005$).

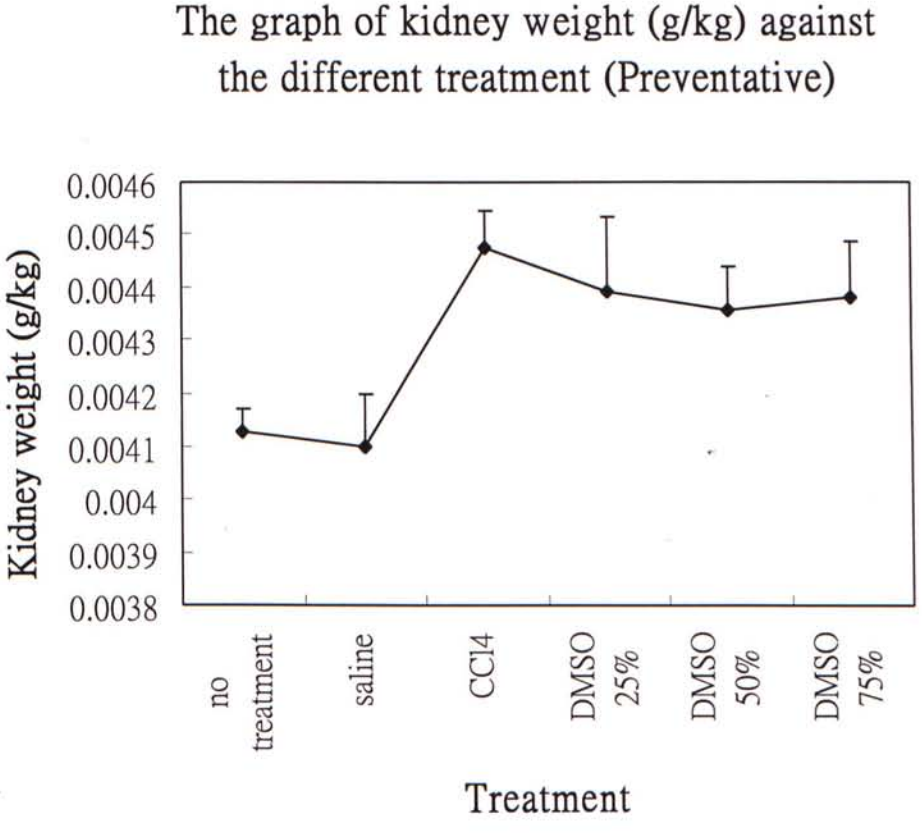


Figure 4.59: Effect of DMSO (at dosages of 25%, 50% and 75%) on CCl_4-induced increase of kidney weight (Preventive). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

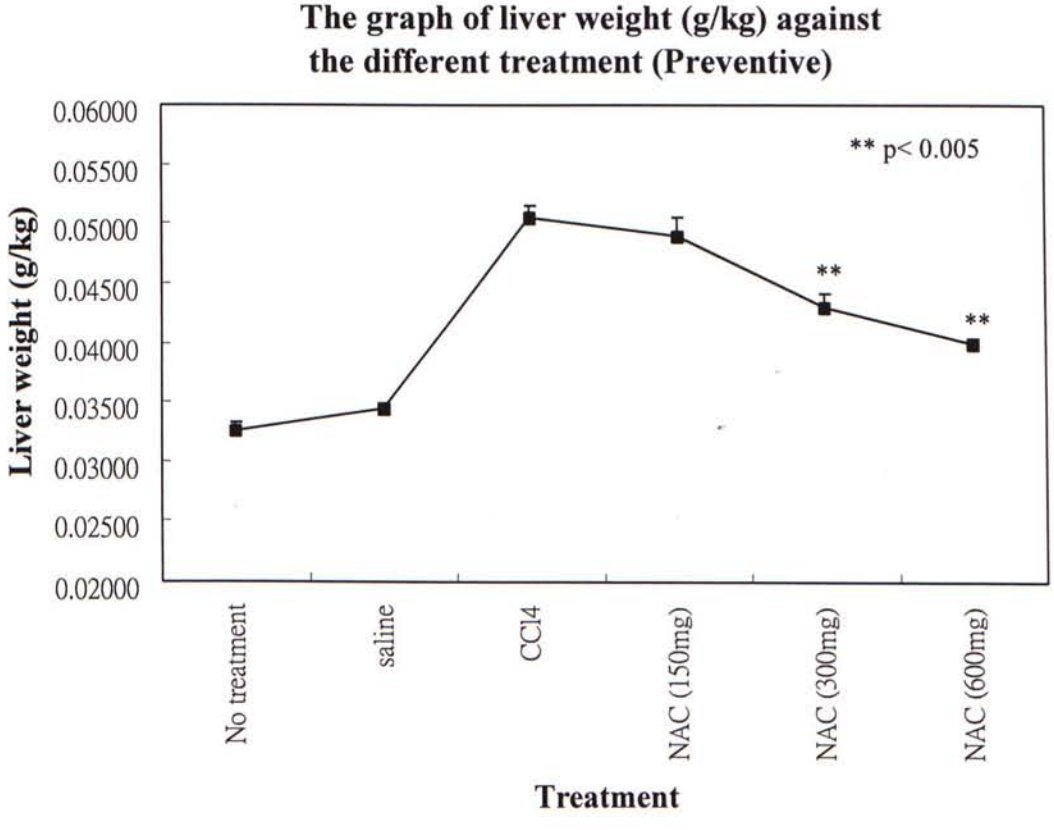


Figure 4.60: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on CCl_4-induced increase of liver weight (Preventive). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by **($p<0.005$).

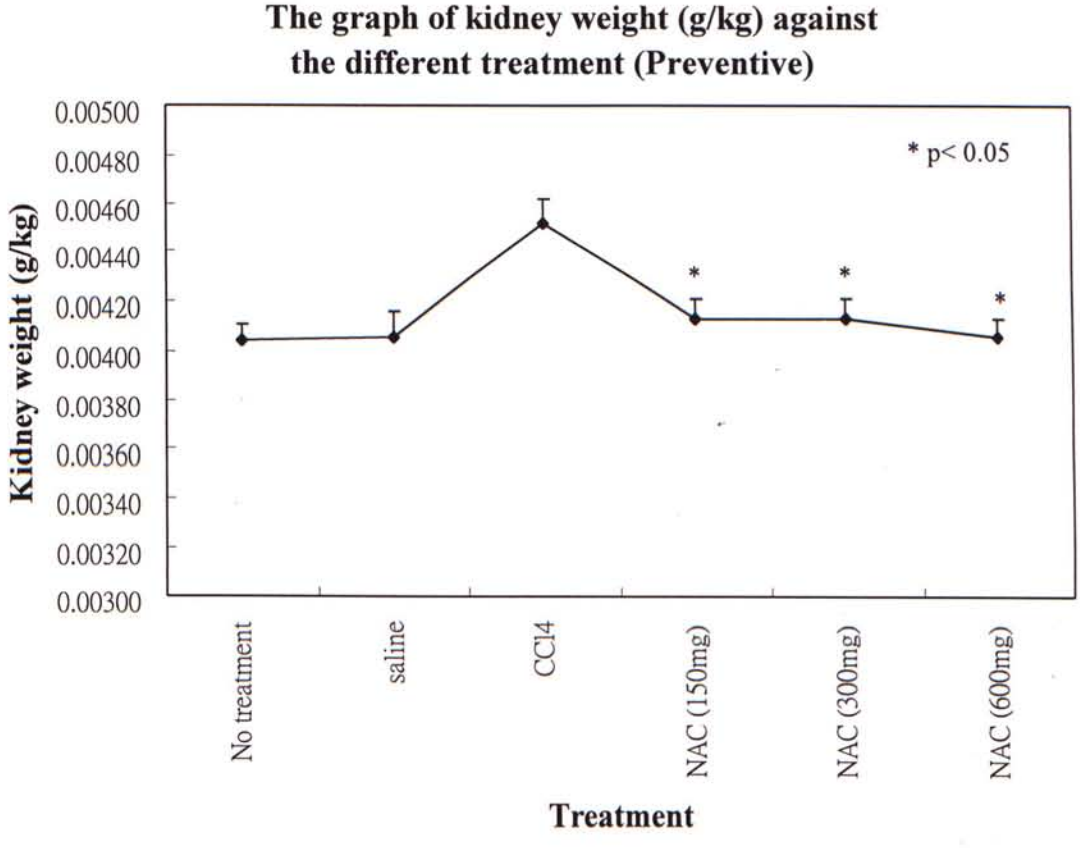


Figure 4.61: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on CCl_4-induced increase of kidney weight (Curative). Each value represents the mean±S.E.M. of 7 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$).

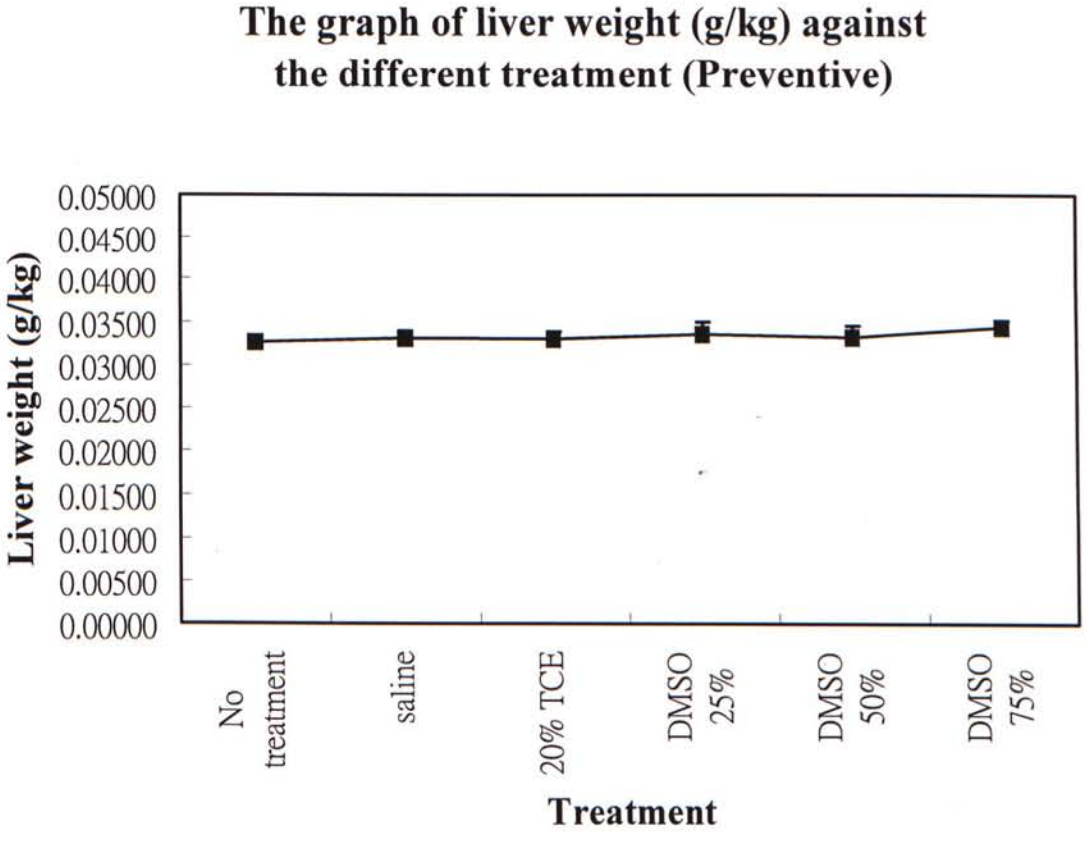


Figure 4.62: Effect of DMSO (at dosages of 25%, 50% and 75%) on liver weight of TCE-treated rats (Preventive). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

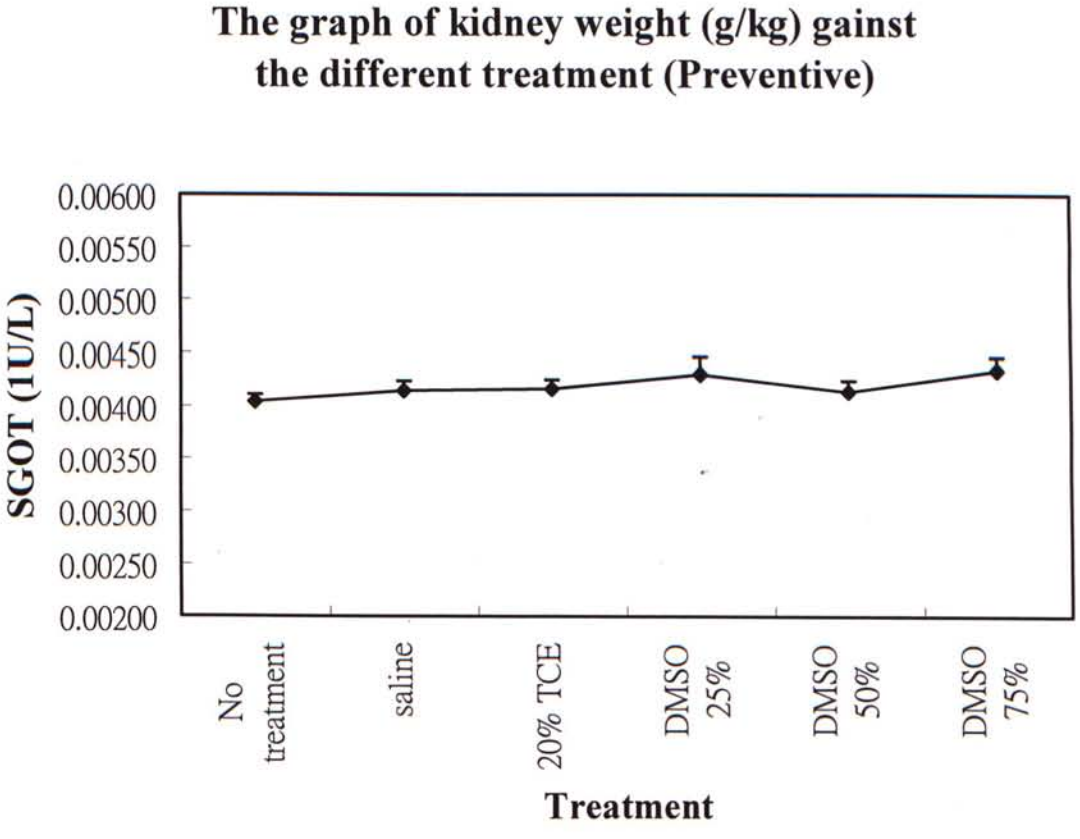


Figure 4.63: Effect of DMSO (at dosages of 25%, 50% and 75%) on kidney weight of TCE-treated rats (Preventive). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

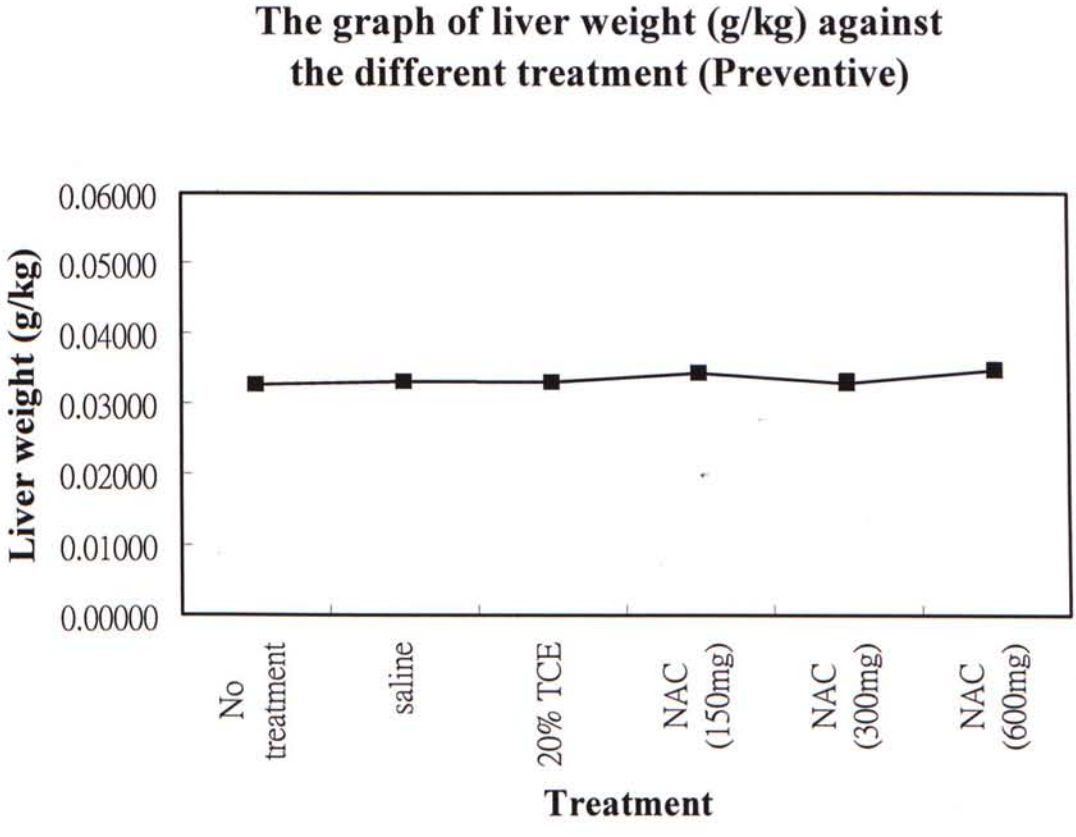


Figure 4.64: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on liver weight of TCE-treated rats (Preventive). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

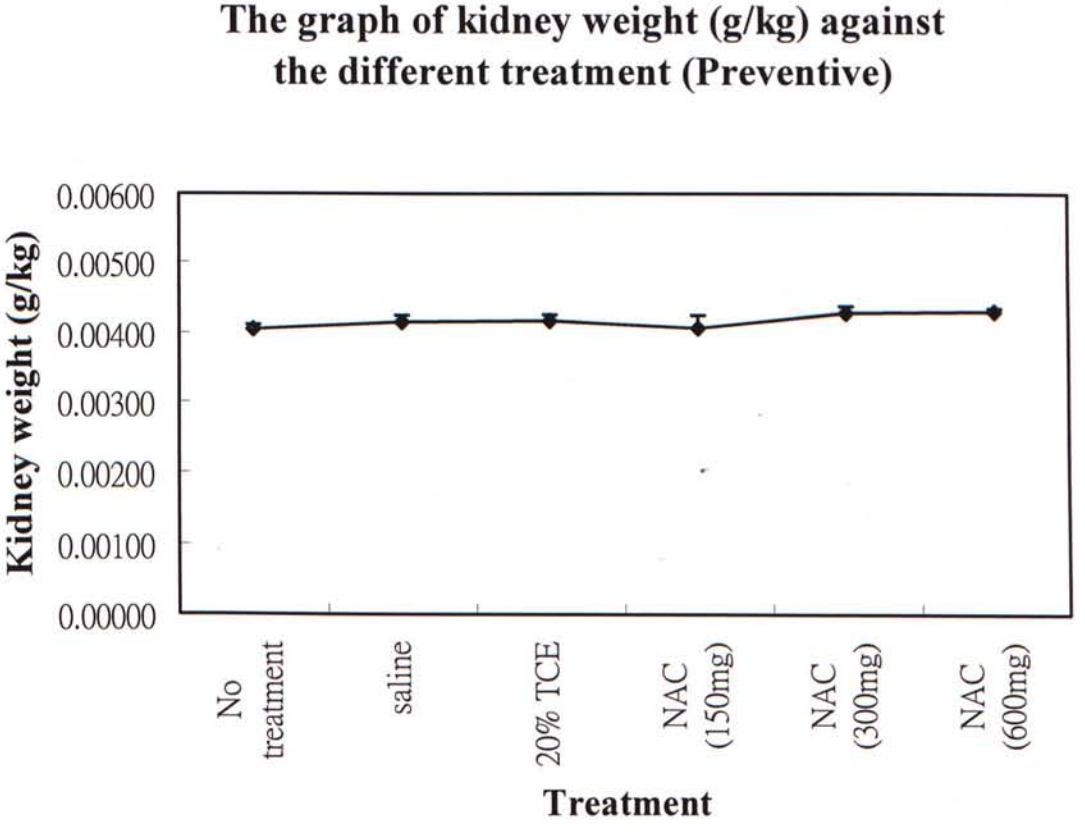


Figure 4.65: Effect of NAC (at dosages of 150 mg/kg, 300 mg/kg and 600 mg/kg) on kidney weight of TCE-treated rats (Preventive). Each value represents the mean±S.E.M. of 6 treated rats except no treatment and control groups which have 5 treated rats in each group. Values statistically significantly different from that of toxin control group are not noted.

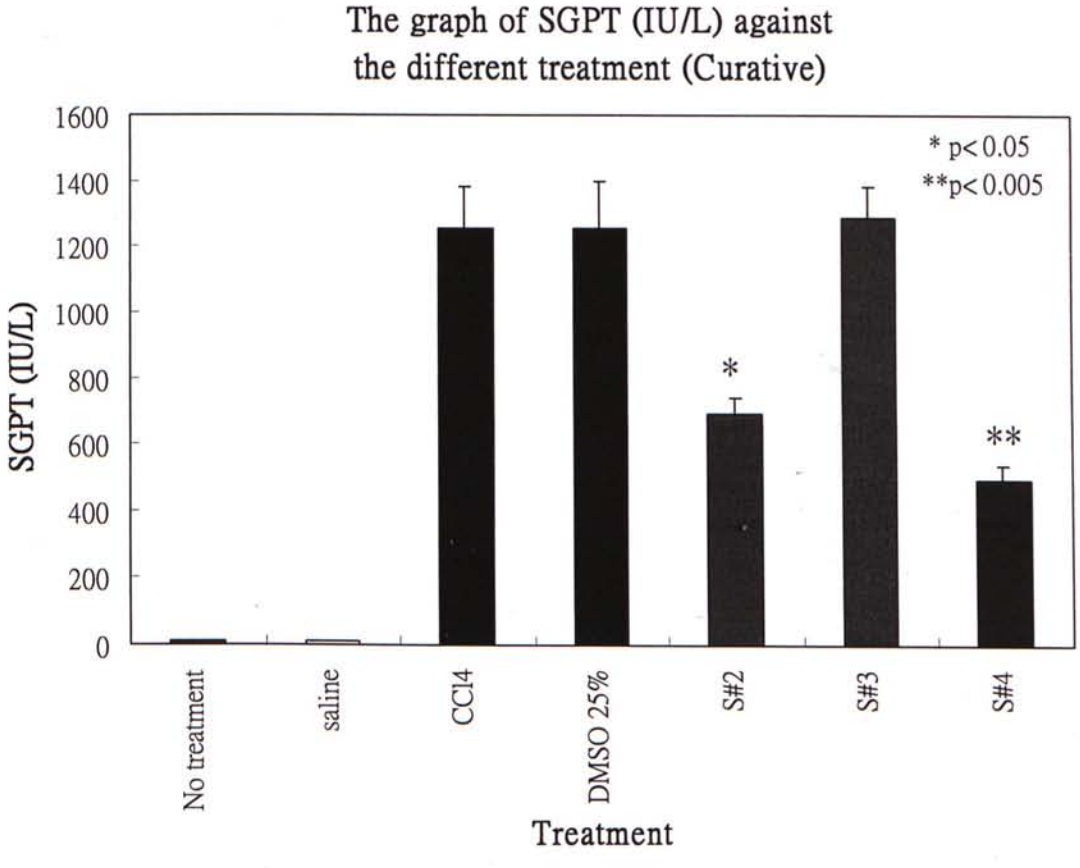


Figure 4.66: Effect of methanol extract (at dosage of 300 mg/kg) of three species of seaweed on CCl_4-induced elevation of SGPT activity (Curative). Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and ** ($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

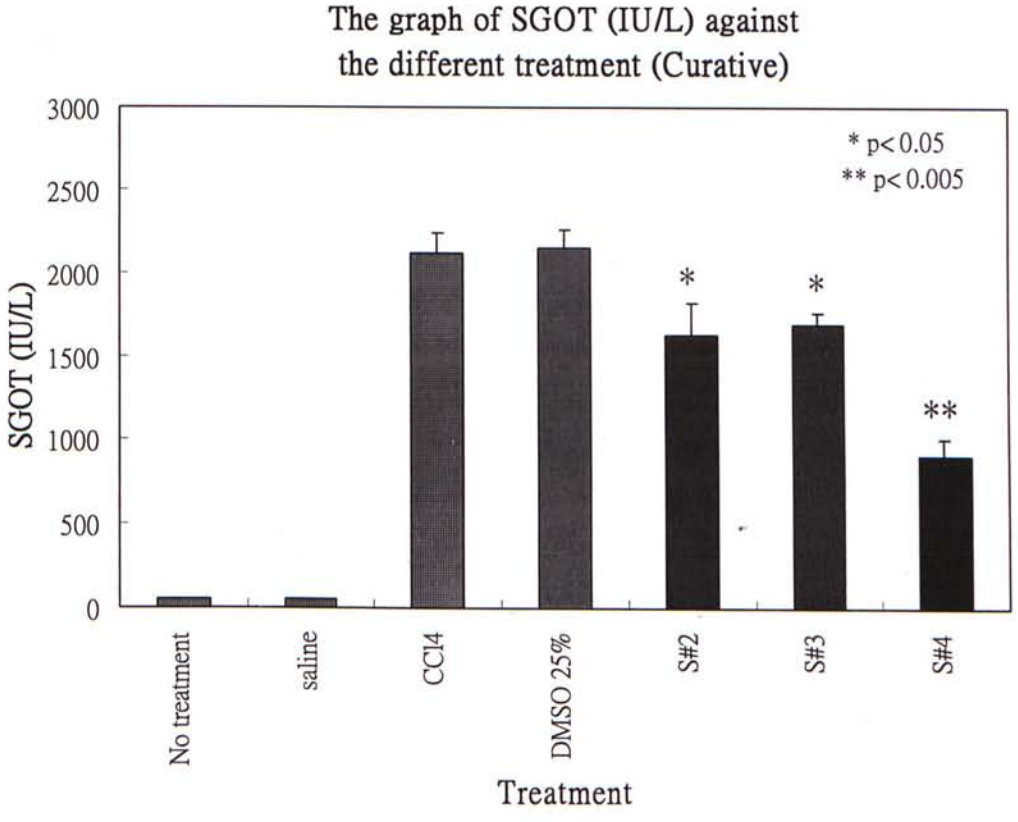


Figure 4.67: Effect of methanol extract (at dosage of 300 mg/kg) of three species of seaweed on CCl_4-induced elevation of SGOT activity (Curative). Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

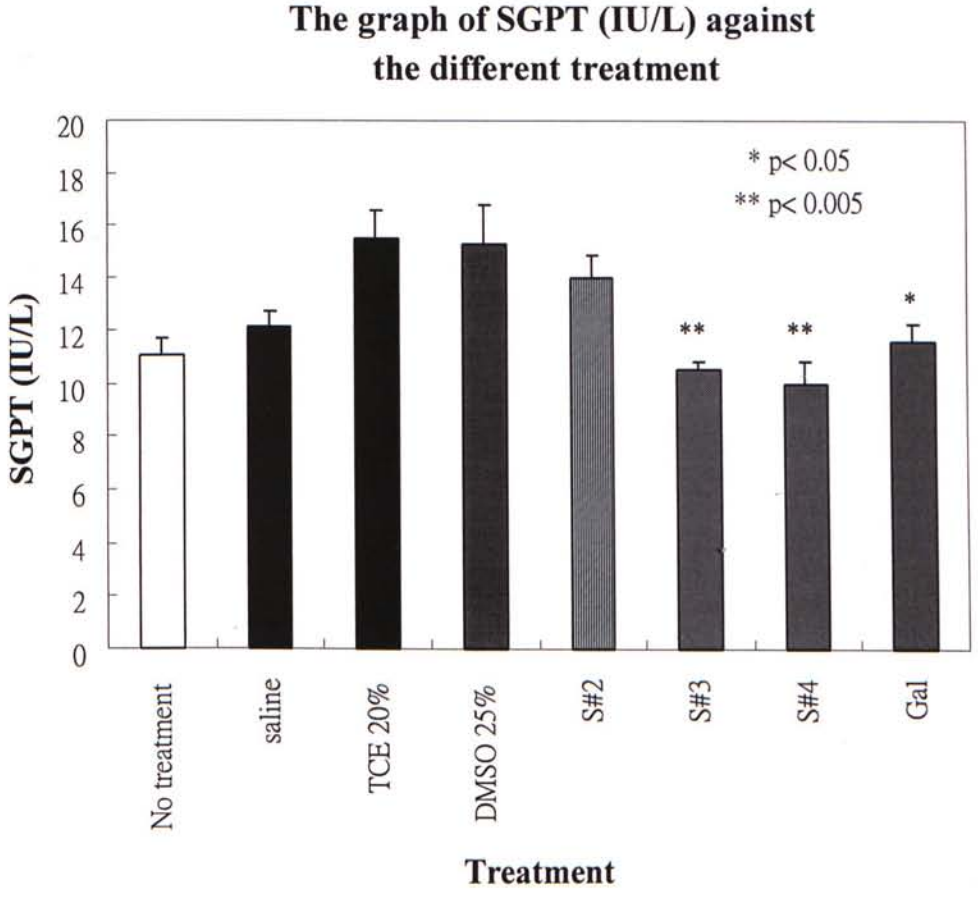


Figure 4.68: Effect of methanol extract (at dosage of 300 mg/kg) of three species of seaweed on TCE-induced elevation of SGPT activity (Curative). Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and ** ($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

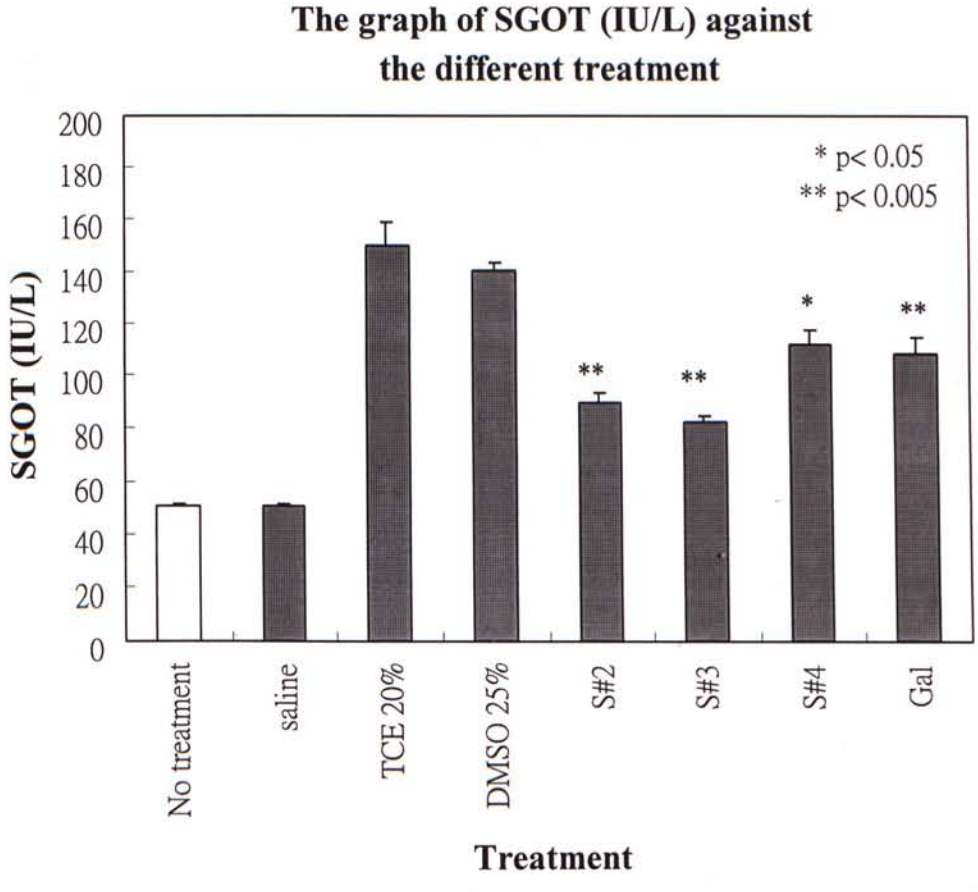


Figure 4.69: Effect of methanol extract (at dosage of 300 mg/kg) of three species of seaweed on TCE-induced elevation of SGOT activity (Curative). Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

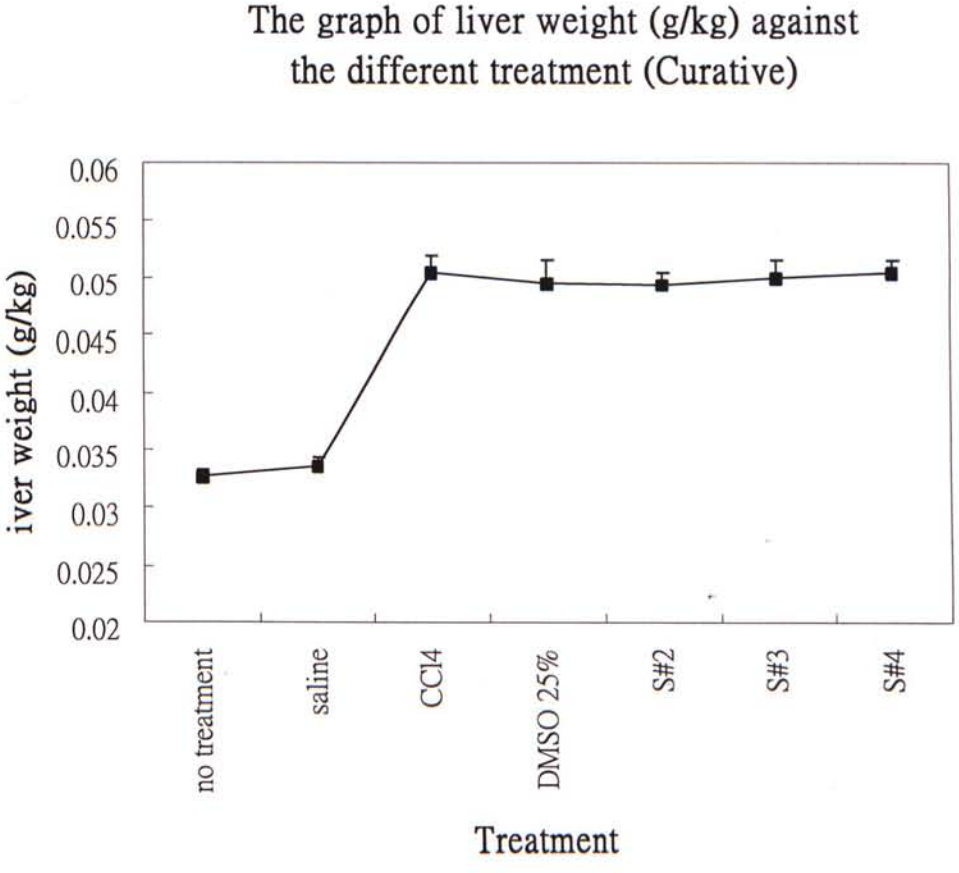


Figure 4.70: Effect of methanol extract (at dosage of 300 mg/ kg) of three species of seaweed on CCl_4-induced increase of liver weight (Curative). Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

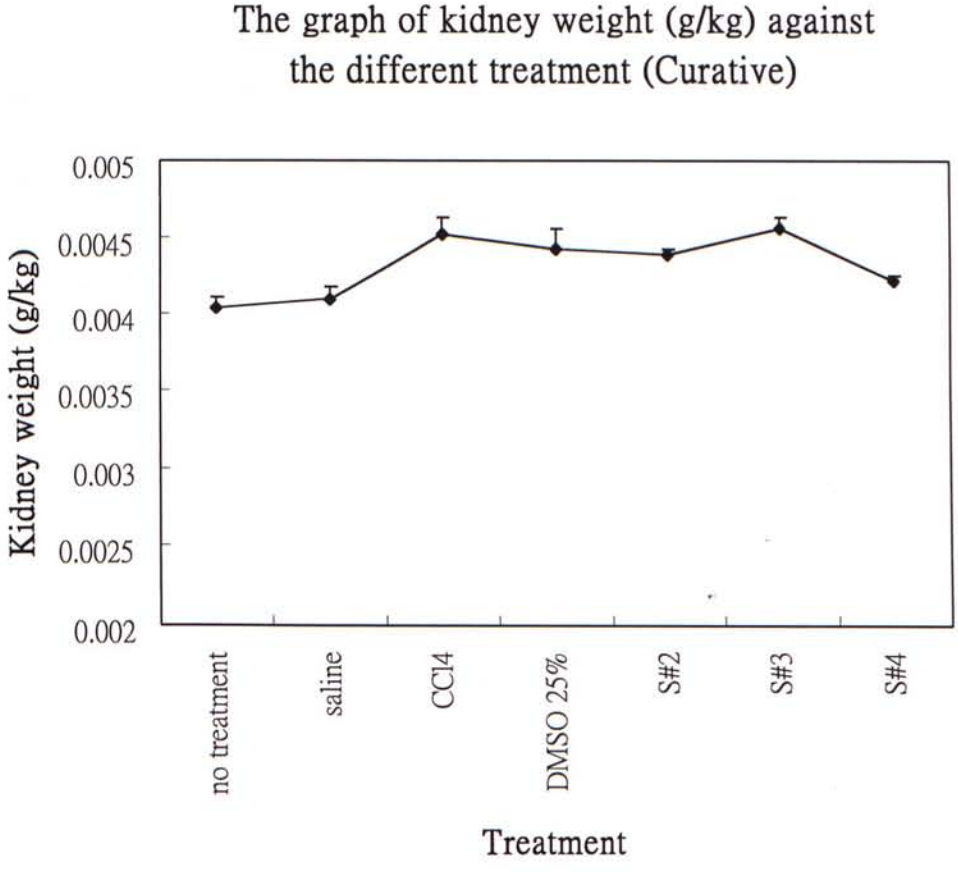


Figure 4.71: Effect of methanol extract (at dosage of 300 mg/ kg) of three species of seaweed on CCl_4-induced increase of kidney weight (Curative). Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of toxin control group are indicated by * ($p<0.05$) and **($p<0.005$).

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*

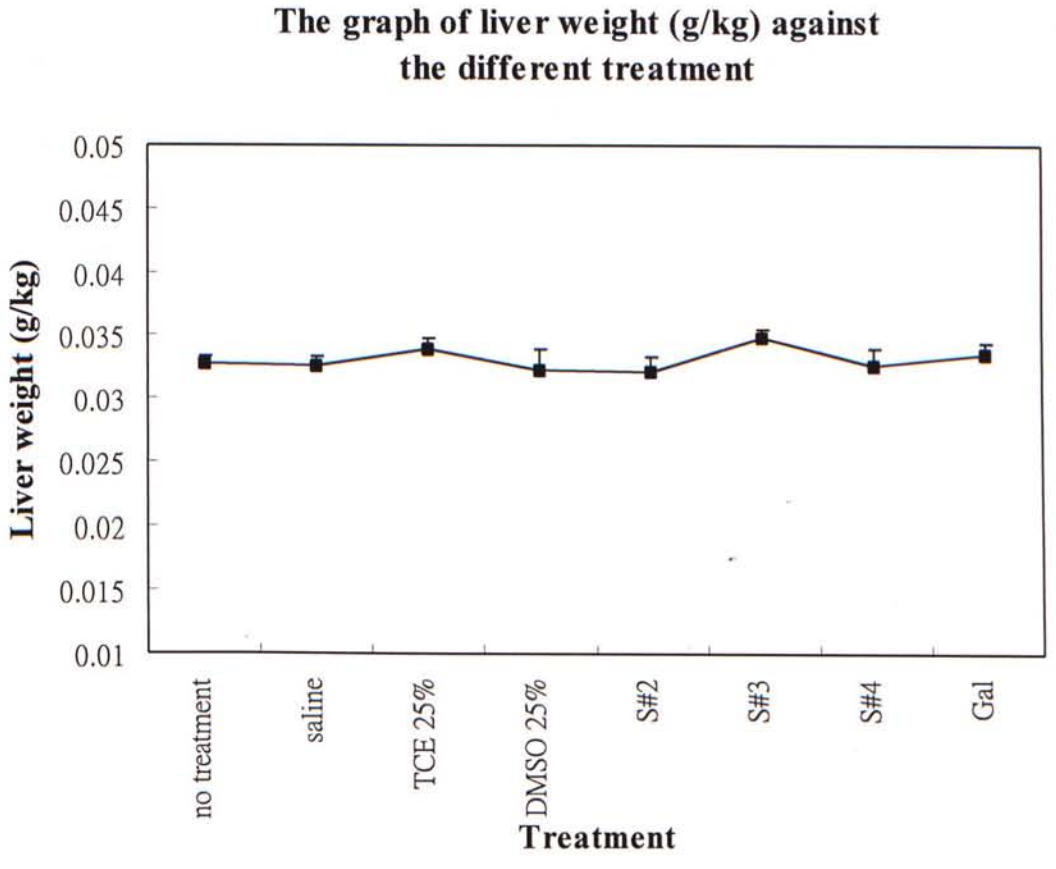


Figure 4.72: Effect of methanol extract (at dosage of 300 mg/ kg) of three species of seaweed on liver weight of TCE-treated rats (Curative). Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of toxin control group are not noted.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

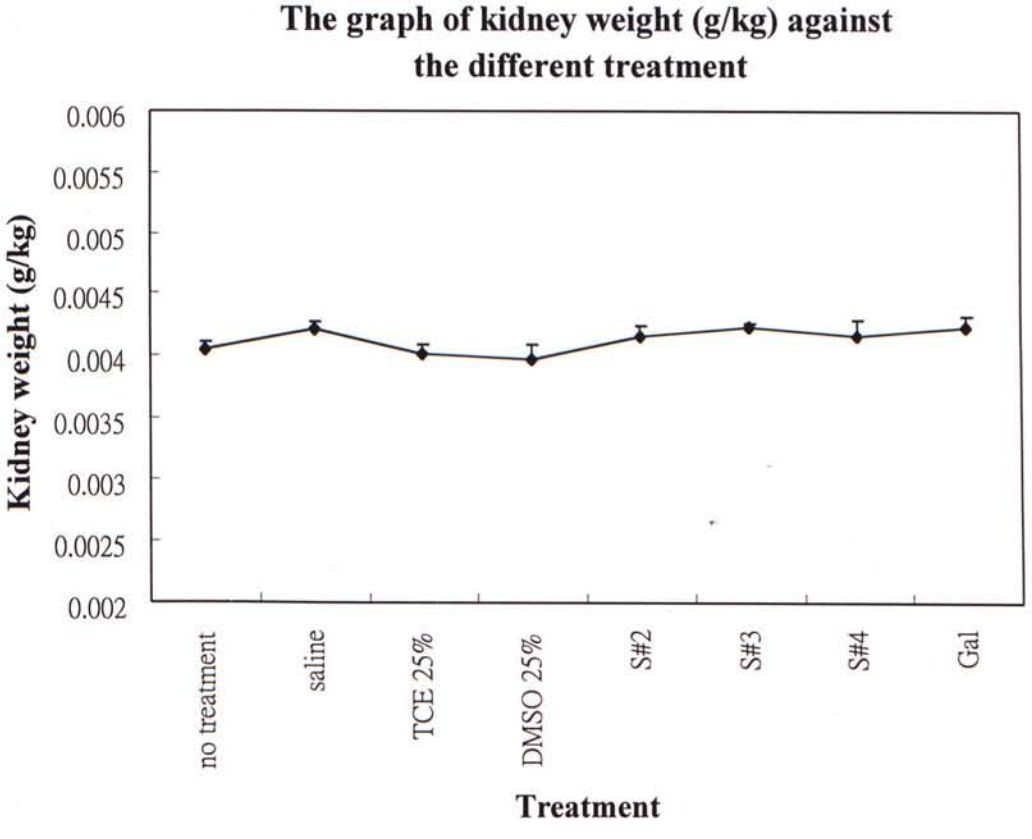


Figure 4.73: Effect of methanol extract (at dosage of 300 mg/ kg) of three species of seaweed on kidney weight of TCE-treated rats (Curative). Each value represents the mean±S.E.M. of 5 treated rats. Values statistically significantly different from that of toxin control group are not noted.

Key: S#2= *Sargassum henslowianum*; S#3= *Myagropsis myagroides*; S#4= *Sargassum siliquastrum*; Gal= *Galaxaura* sp.

Fig. 4.74 Micrograph of the liver of rat from no treatment group showing normal hepatocytes around the central vein region. cv, central vein; p, portal triad.
(60 x, H & E)

Fig. 4.75 Micrograph of the liver of rats from no treatment group showing normal hepatocytes around the central vein region. cv, central vein; si, sinusoid; p, portal triad.
(148x, H & E)


p
cv

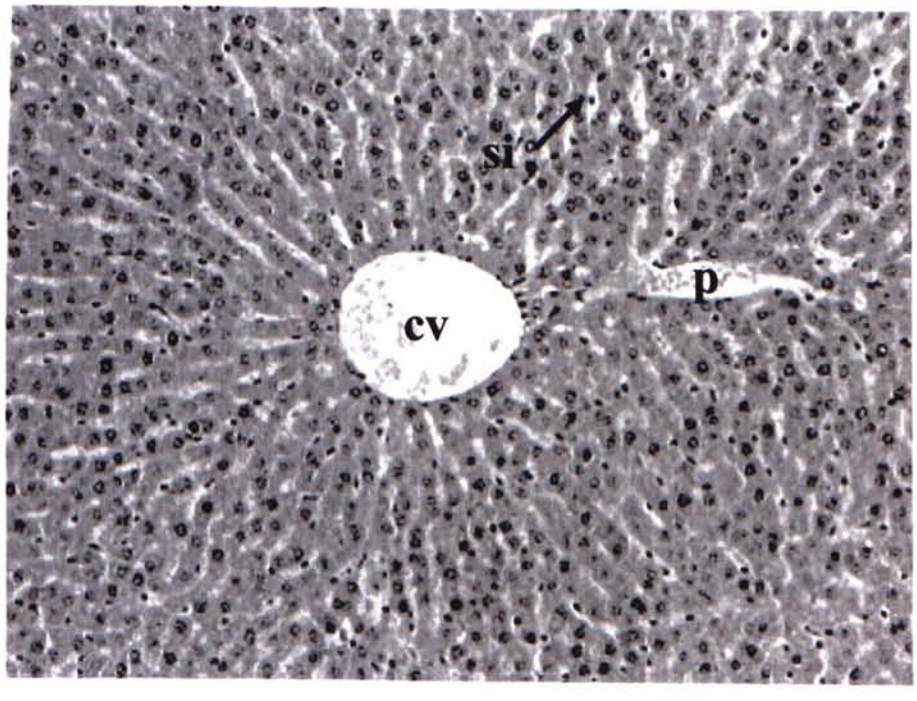
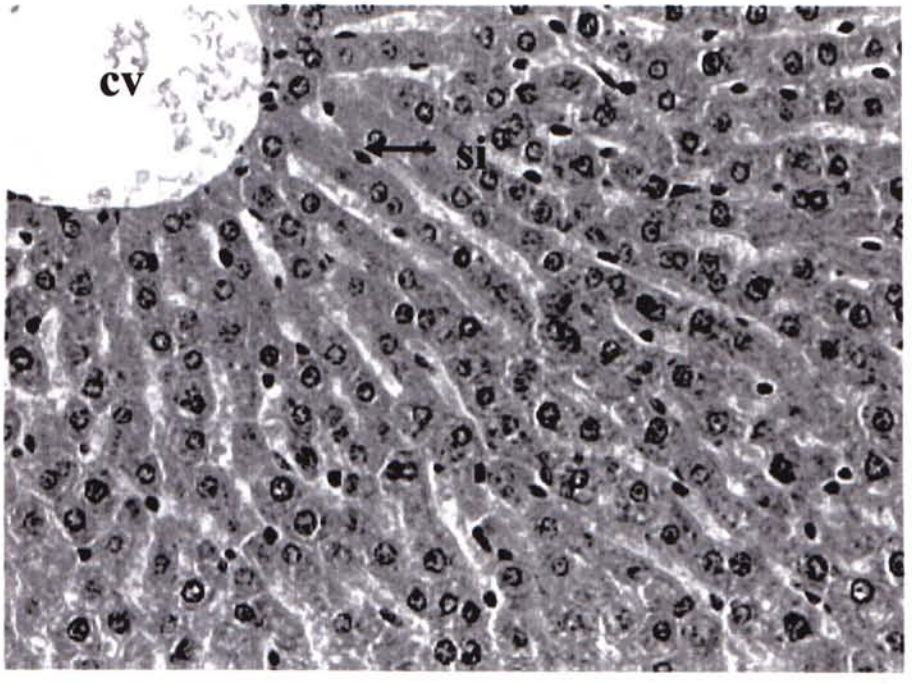

si
p
cv

Fig. 4.76 Micrograph of the liver of rat from no treatment group showing normal hepatocytes around the central vein region. cv, central vein; si, sinusoid; p, portal triad.
(587 ×, H & E)

Fig. 4.77 Micrograph of the liver of rat from vehicle-saline curative group showing normal hepatocytes around the central vein region. cv, central vein; p, portal triad.
(40 ×, H & E)


cv
si

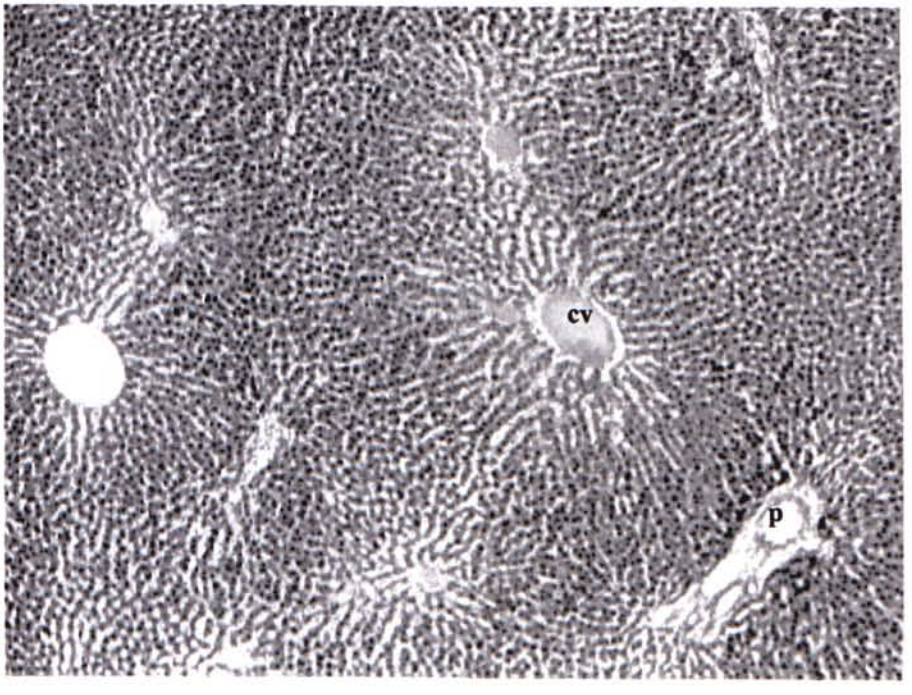
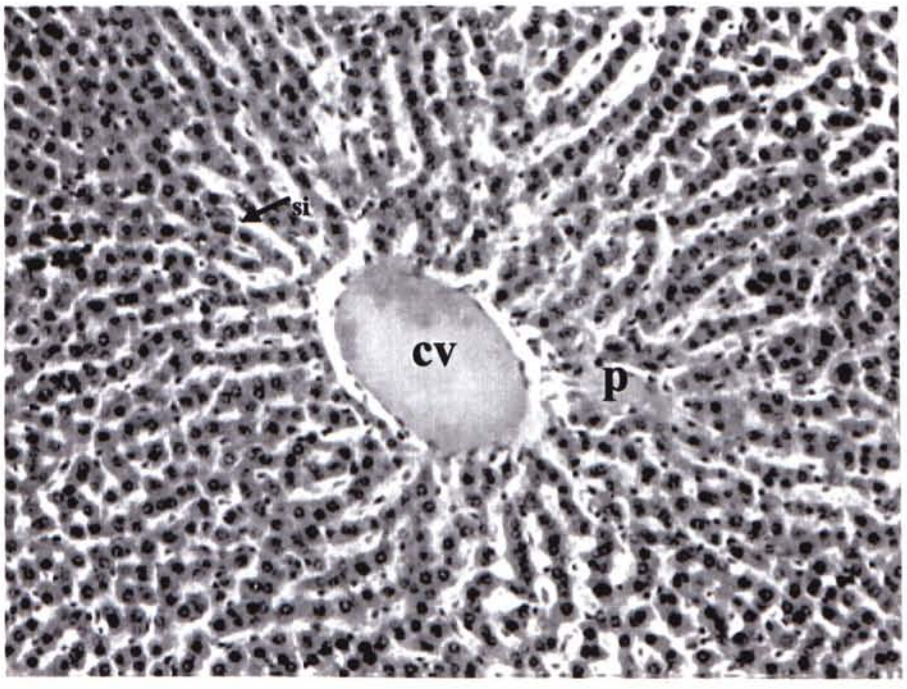

cv
p

Fig. 4.78 Micrograph of liver of rat from the vehicle-saline curative group showing normal hepatocytes around the central vein region. cv, central vein; p, portal triad; si, sinusoid.
(147x, H & E)

Fig. 4.79 Micrograph of the liver of rat from the vehicle-saline curative group showing normal hepatocytes around the central vein region. cv, central vein; p, portal triad; si, sinusoid.
(293 x, H & E)


si
cv
p

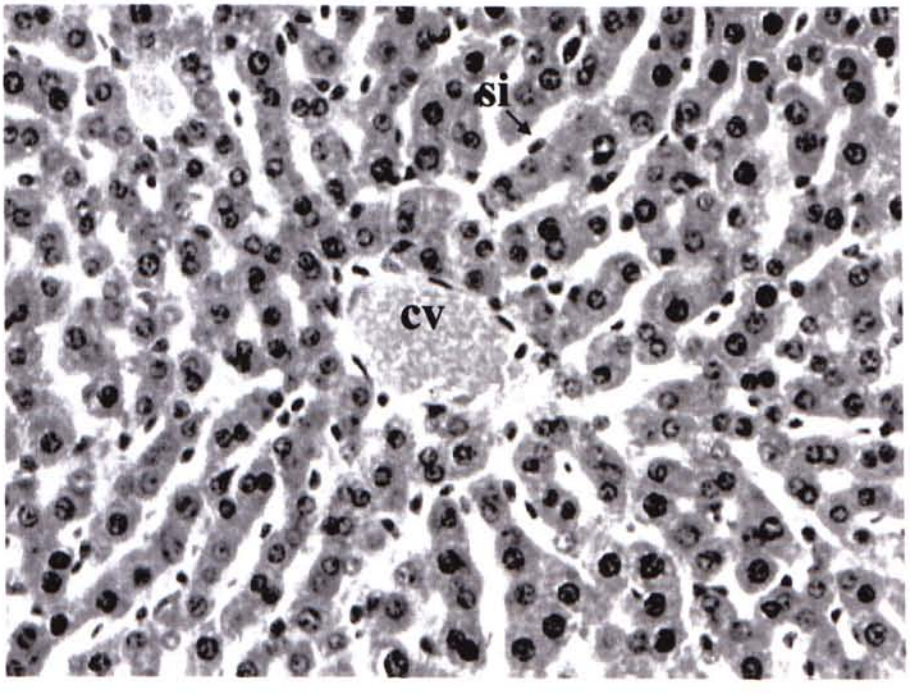
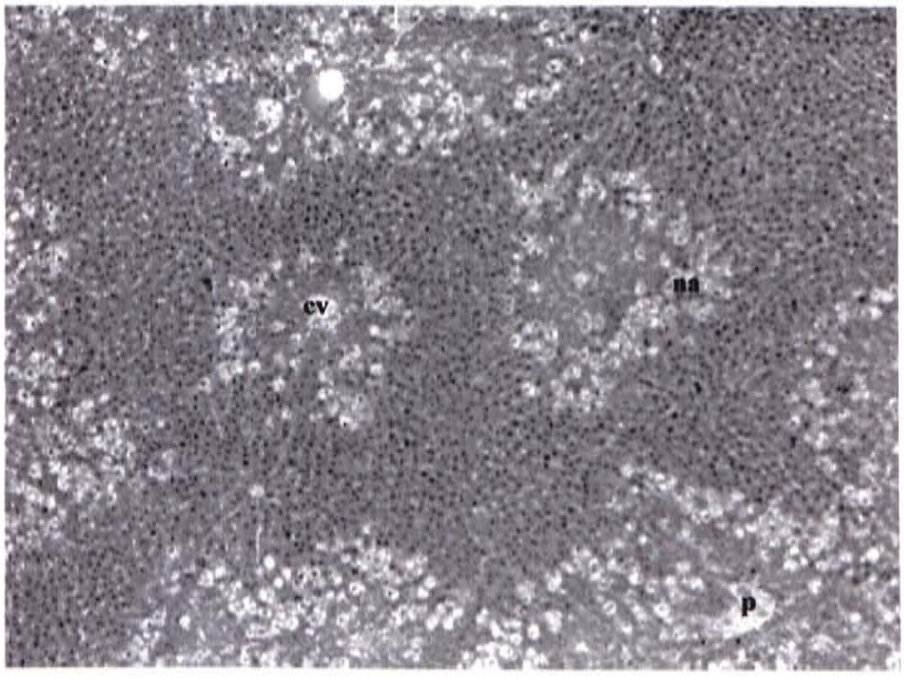

si
cv

Fig. 4.80 Micrograph of the liver of CCl_4-treated rat from toxin control curative group showing extensive necrosis of hepatocytes around the central vein region as compared with the control group. cv, central vein; na, necrotic area; p, portal triad.
(60 ×, H & E)

Fig. 4.81 Micrograph of the liver of CCl_4-treated rat from toxin control curative group showing extensive necrosis of hepatocytes around the central vein region as compared with the control group. cn, condensed nucleus; cv, central vein; na, necrotic area; sc, swellen cell; v, vacuolation
(148 ×, H & E)


cv
na
p

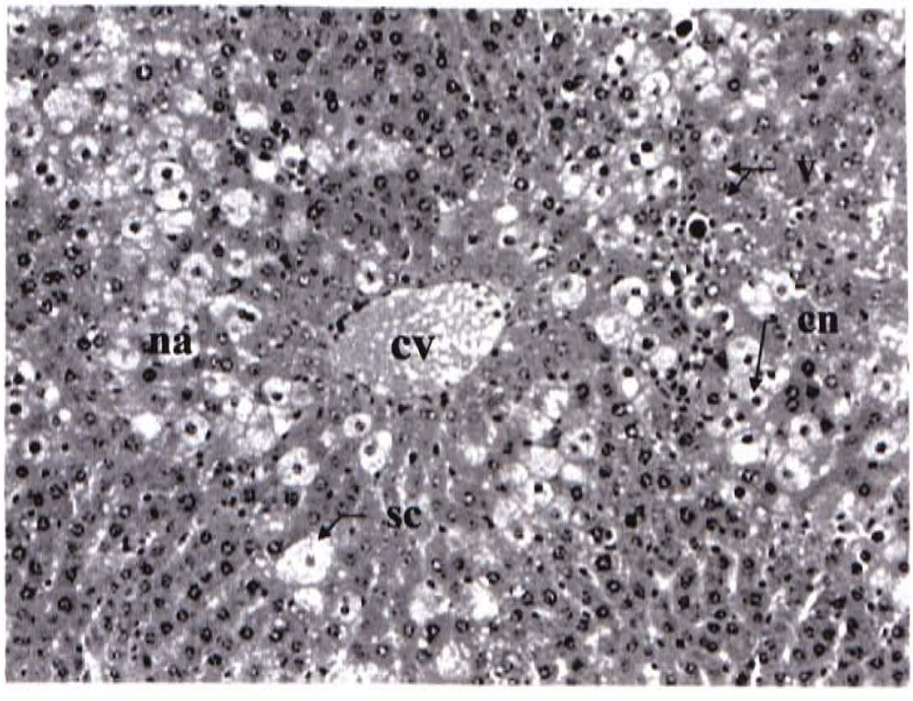
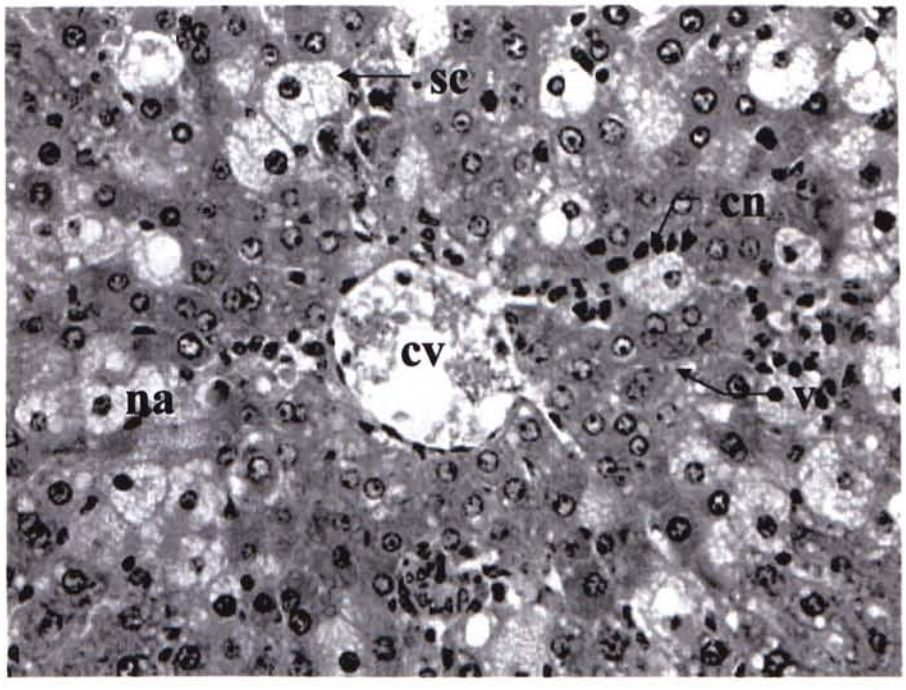

V
cn
na
cv
sc

Fig. 4.82 Micrograph of the liver of CCl_4-treated rat from toxin control curative group showing extensive necrosis of hepatocytes around the central vein region as compared with the control group. cn, condensed nucleus; cv, central vein; na, necrotic area; sc, swellen cell; v, vacuolation
(293 ×, H & E)

Fig. 4.83 Effect of seaweed extract (S#2: *S. henslowianum*, 15 mg/ml saline) on the liver of CCl_4-treated rat showing extensive necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cn, condensed nucleus; cv, central vein; sc, swellen cell; v, vacuolation.
(97 ×, H & E)


sc
cn
cv
na
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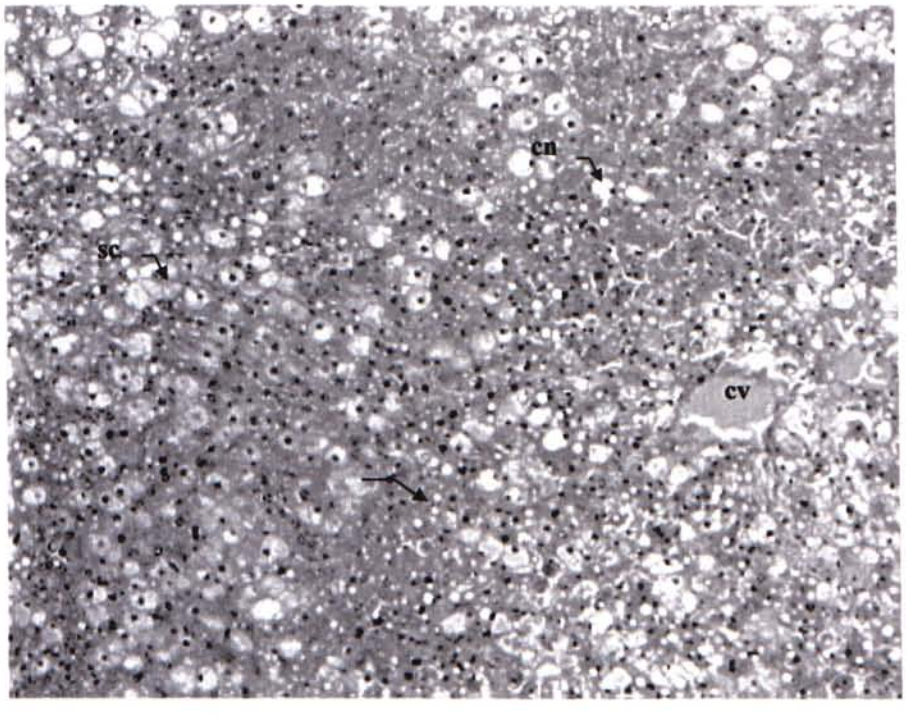
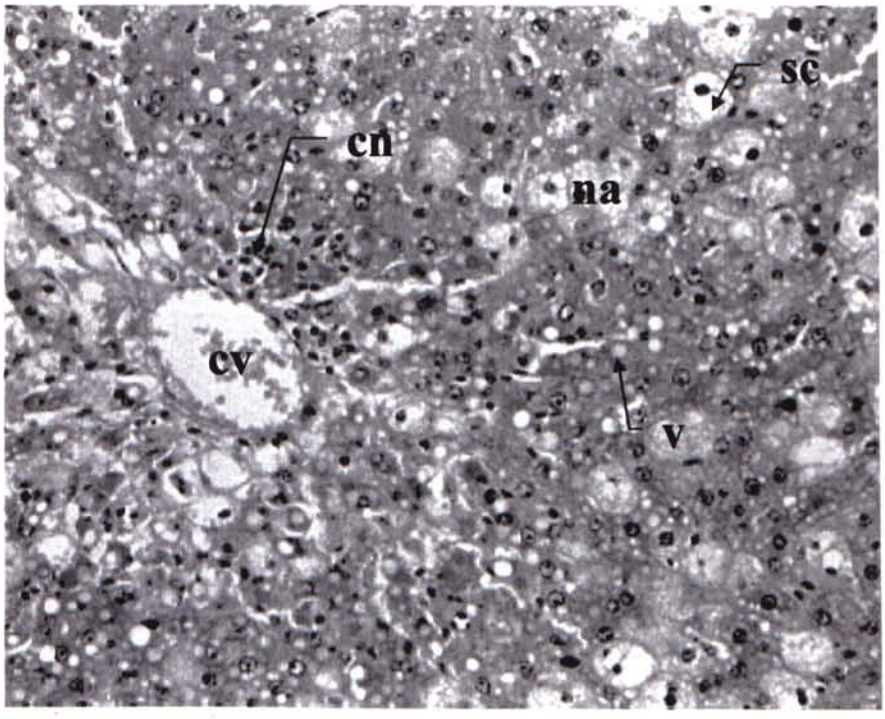

cn
sc
cv

Fig. 4.84 Effect of seaweed extract (S#2: *S. henslowianum*, 15 mg/ml saline) on the liver of CCl_4-treated rat showing extensive necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cn, condensed nucleus; cv, central vein; na, necrotic area; sc, swellen cell; v, vacuolation.
(194 ×, H & E)

Fig. 4.85 Effect of seaweed extract (S#2: *S. henslowianum*, 60 mg/ml saline) on the liver of CCl_4-treated rat showing very little necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cv, central vein; rz, regeneration zone.
(95 ×, H & E)


sc
cn
na
cv
v

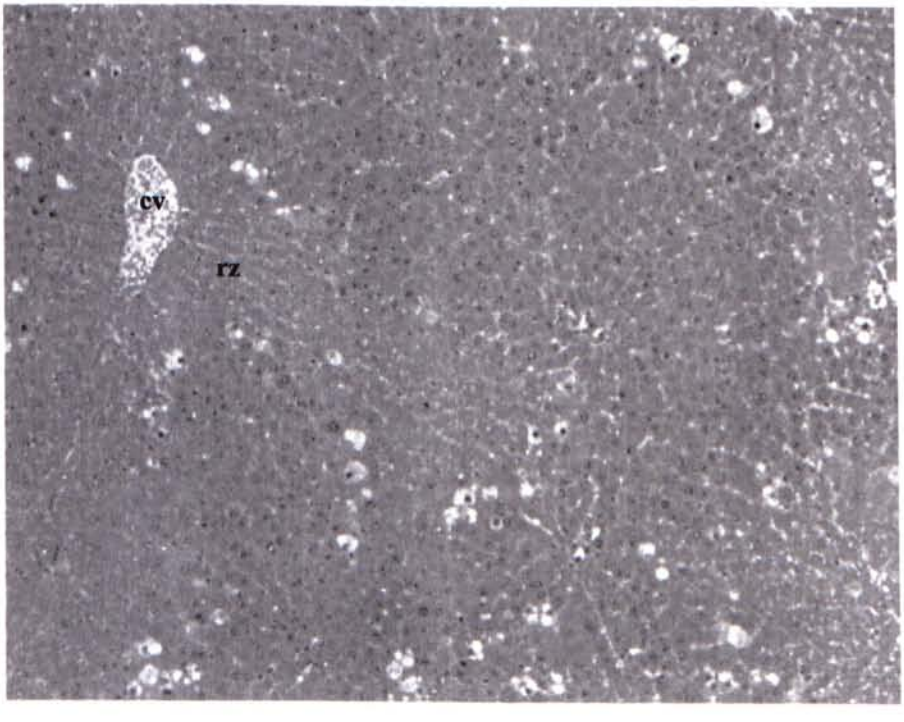
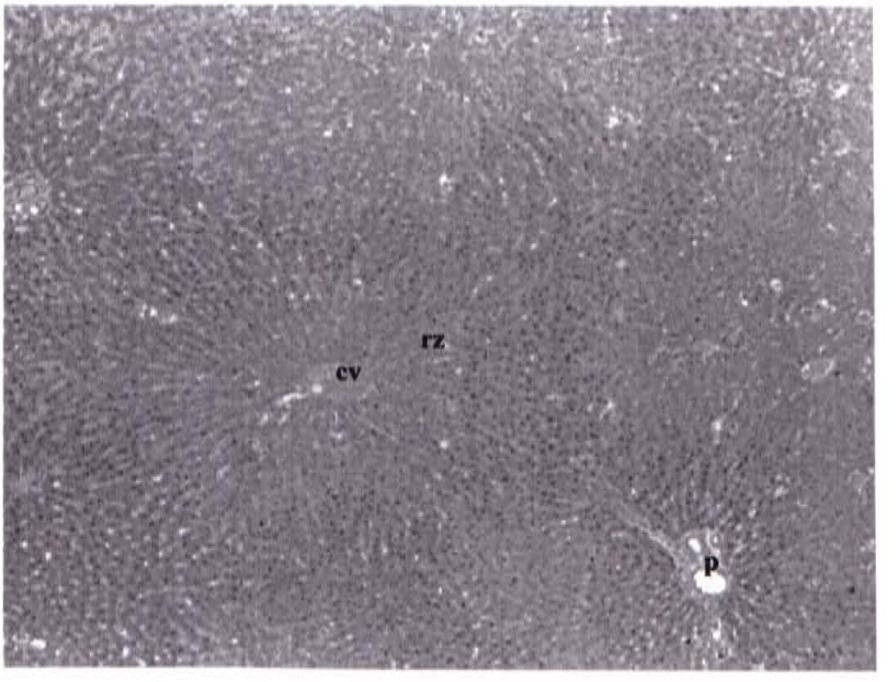

cv
rz

Fig. 4.86 Effect of the seaweed extract (S#2: *S. henslowianum*, 60 mg/ml saline) on the liver of CCl_4-treated rat (duplicated) showing very little necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cv, central vein; p, portal triad; rz, regeneration zone.
(60 ×, H & E)

Fig. 4.87 Effect of the seaweed extract (S#2: *S. henslowianum*, 60 mg/ml saline) on the liver of CCl_4-treated rat (duplicated) showing very little necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. But, vacuolation can still be observed around the central vein. cv, central vein; nc, necrotic cell; rz, regeneration zone; sc, swellen cells; v, vacuolation.
(148 ×, H & E)


rz
cv
p

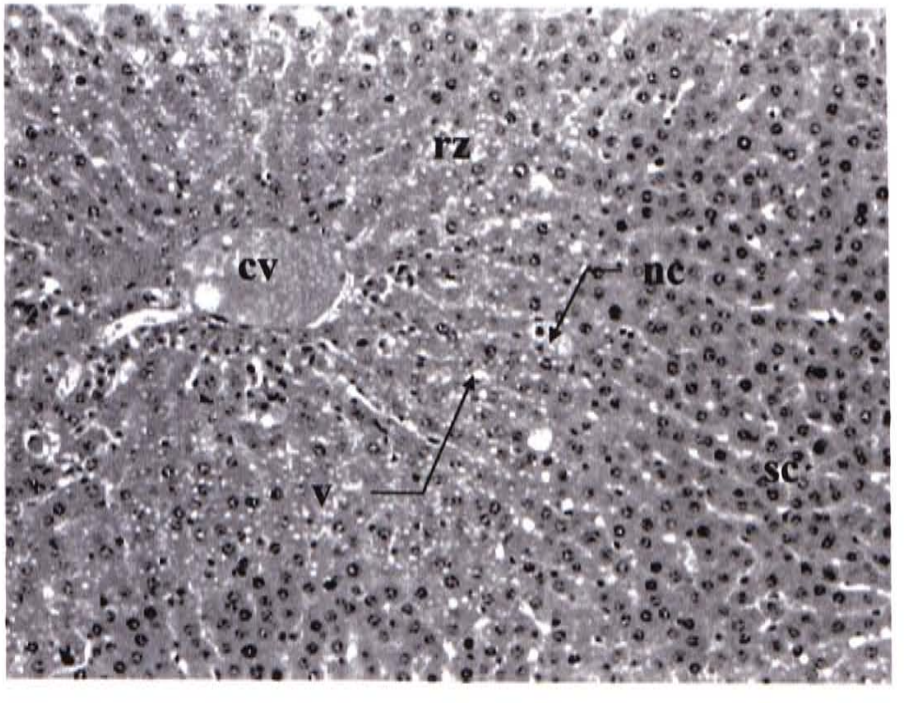
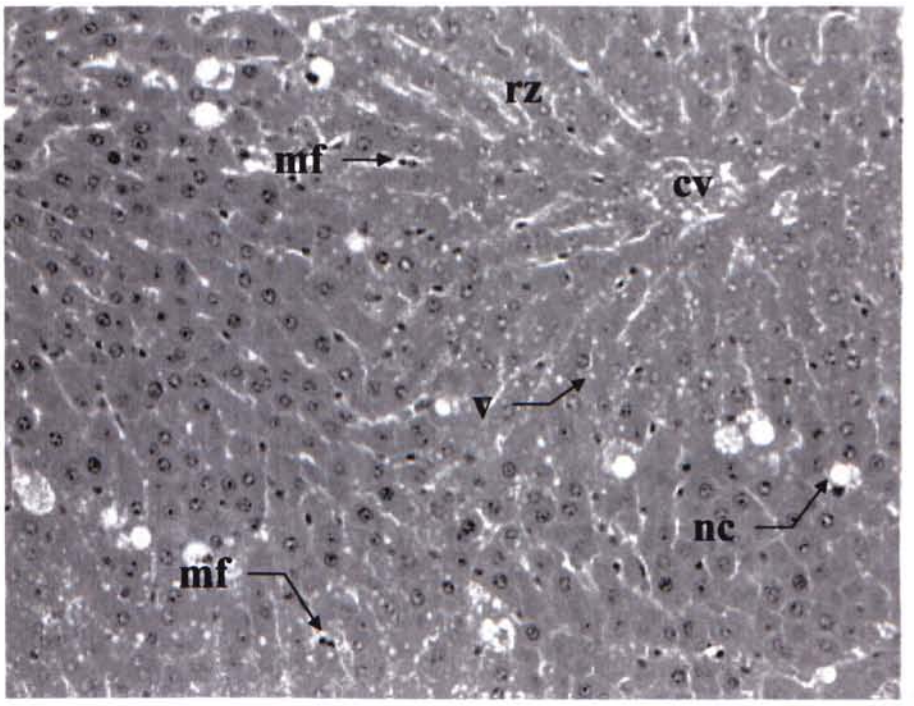

rz
cv
nc
v
sc

Fig. 4.88 Effect of the seaweed extract (S#2: *S. henslowianum*, 60 mg/ml saline) on the liver of CCl_4-treated rat (duplicated) showing very little necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. But, vacuolation can still be observed around the central vein. Large regeneration zone can be seen. cv, central vein; mf, mitotic figure; nc, necrotic cell; rz, regeneration zone; sc, swellen cells; v, vacuolation.
(194 ×, H & E)

Fig. 4.89 Effect of the seaweed extract (S#3: *Myagropsis myagroides*, 15 mg/ml saline) on the liver of CCl_4-treated rat showing extensive necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cv, central vein; na, necrotic area; rz, regeneration zone.
(148 ×, H & E)


rz
mf
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mf

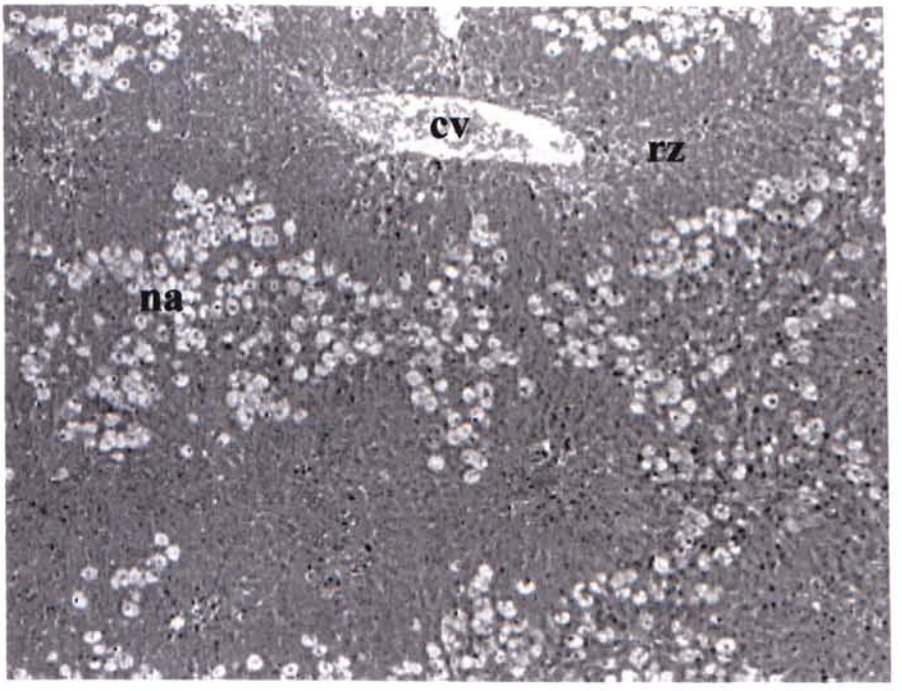
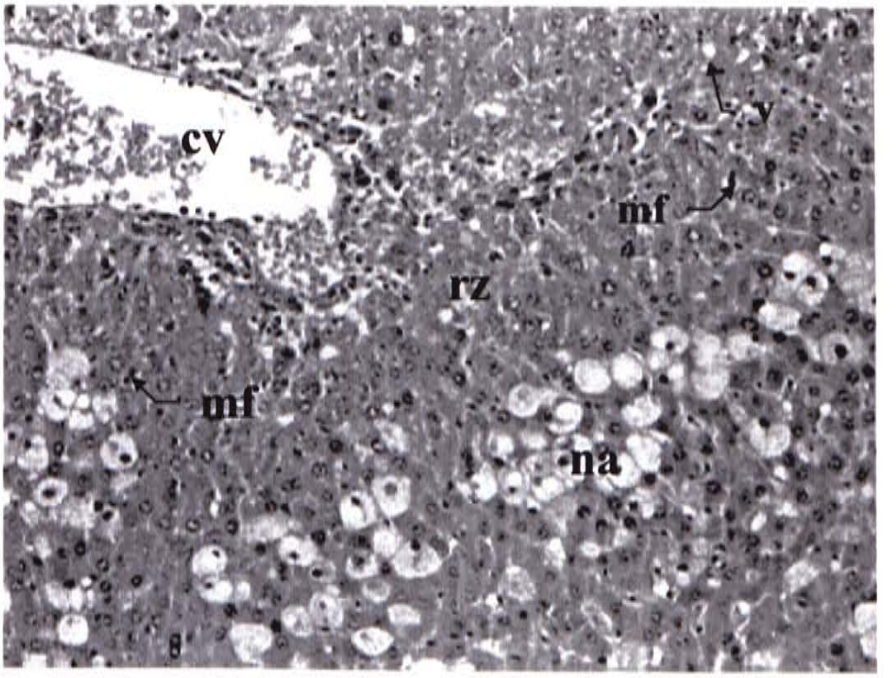

cv
rz
na

Fig. 4.90 Effect of the seaweed extract (S#3: *Myagropsis myagroides*, 15 mg/ml saline) on the liver of CCl_4-treated rat showing extensive necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cv, central vein; mf, mitotic figure; na, necrotic area; rz, regeneration zone; v, vacuolation.
(297 ×, H & E)

Fig. 4.91 Effect of the seaweed extract (S#3: *Myagropsis myagroides*, 30 mg/ml saline) on the liver of CCl_4-treated rat showing few necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. Medium area of regeneration zone can be seen. cv, central vein; pv, hepatic portal vein; rz, regeneration zone.
(60 ×, H & E)


cv
v
mf
rz
mf
na

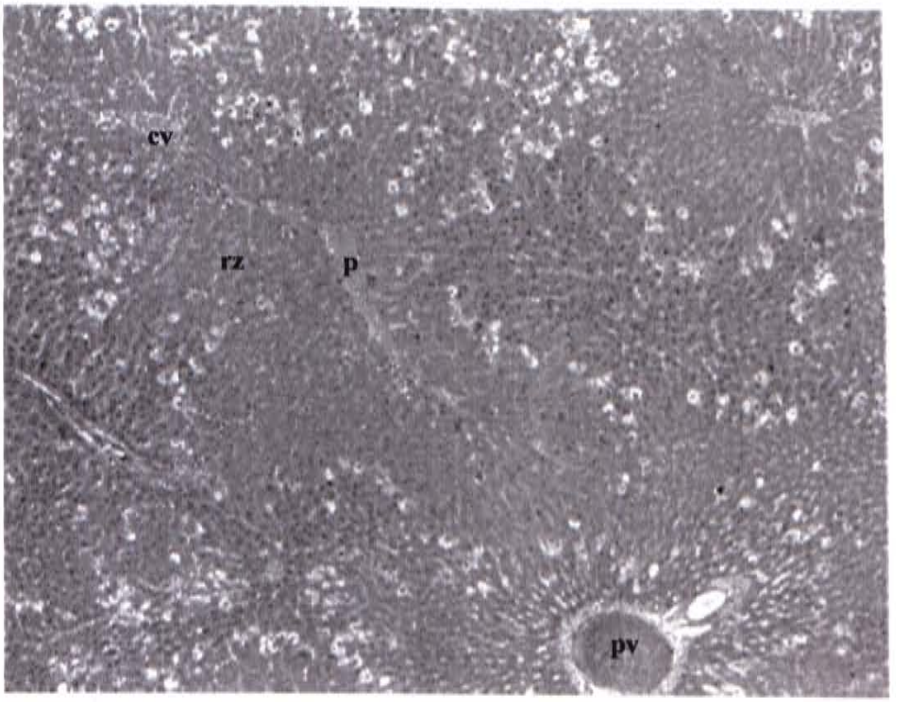
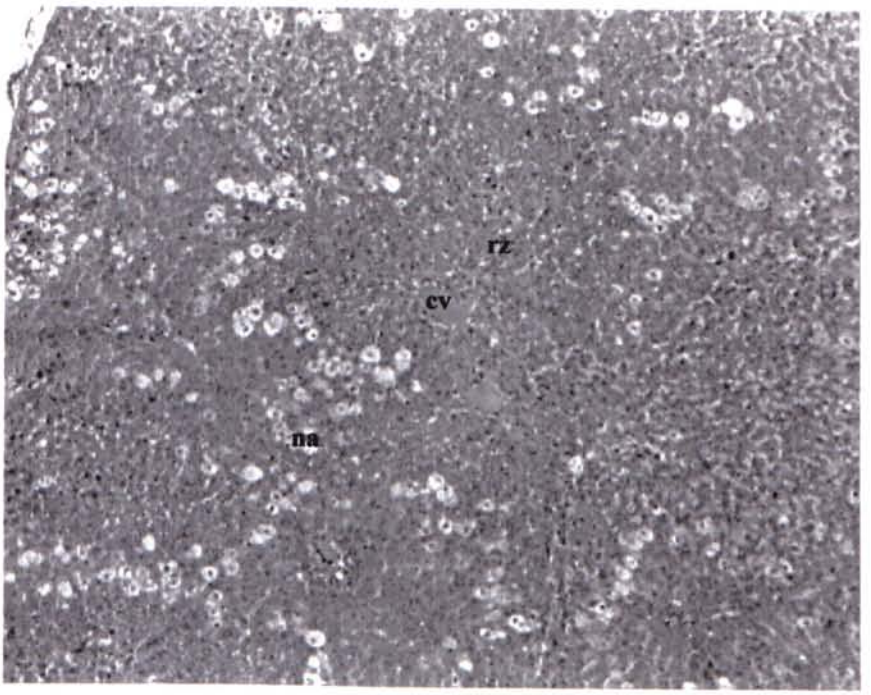

cv
rz
p
pv

Fig. 4.92 Effect of the seaweed extract (S#3: *Myagropsis myagroides*, 60 mg/ml saline) on the liver of CCl_4-treated rat showing few necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. Large area of regeneration zone can be seen. cv, central vein; na, necrotic area; rz, regeneration zone.
(60 x, H & E)

Fig. 4.93 Effect of the seaweed extract (S#3: *Myagropsis myagroides*, 60 mg/ml saline) on the liver of CCl_4-treated rat showing few necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. Large area of regeneration zone can be seen. cv, central vein; mf, mitotic figure; na, necrotic area; rz, regeneration zone; v, vacuolization.
(148 x, H & E)


rz
cv
na

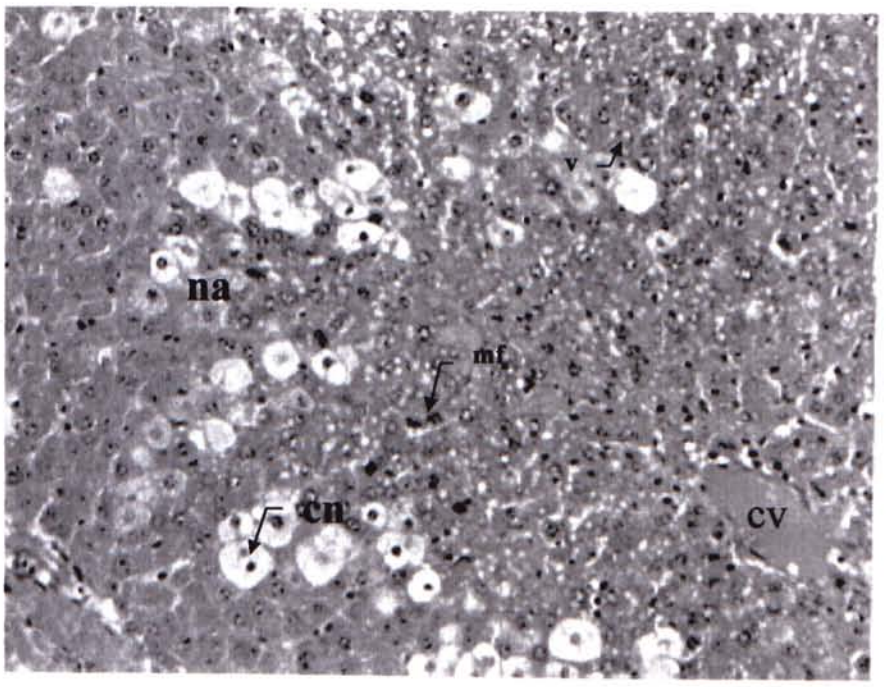
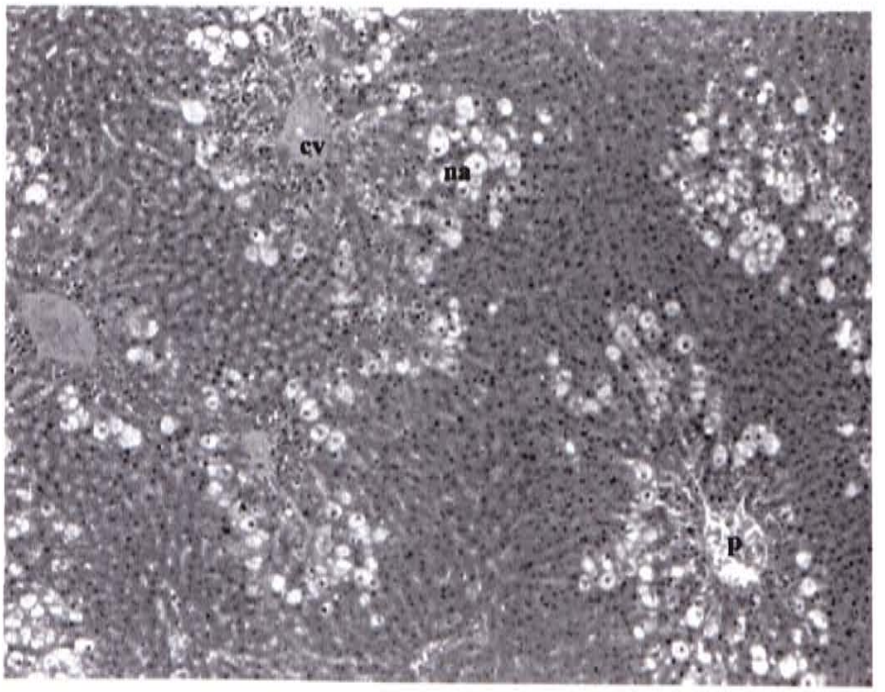

v
na
mf
cn
CV

Fig. 4.94 Effect of the seaweed extract (S#4: *S. siliquastrum*, 15 mg/ml saline) on the liver of CCl_4-treated rat showing extensive necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cv, central vein; na, necrotic area; p, portal triad.
(60 ×, H & E)

Fig. 4.95 Effect of the seaweed extract (S#4: *S. siliquastrum*, 15 mg/ml saline) on the liver of CCl_4-treated rat showing extensive necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cv, central vein; cn, condensed nucleus; nc, necrotic cell; v, vacuolization.
(297 ×, H & E)


cv
na
p

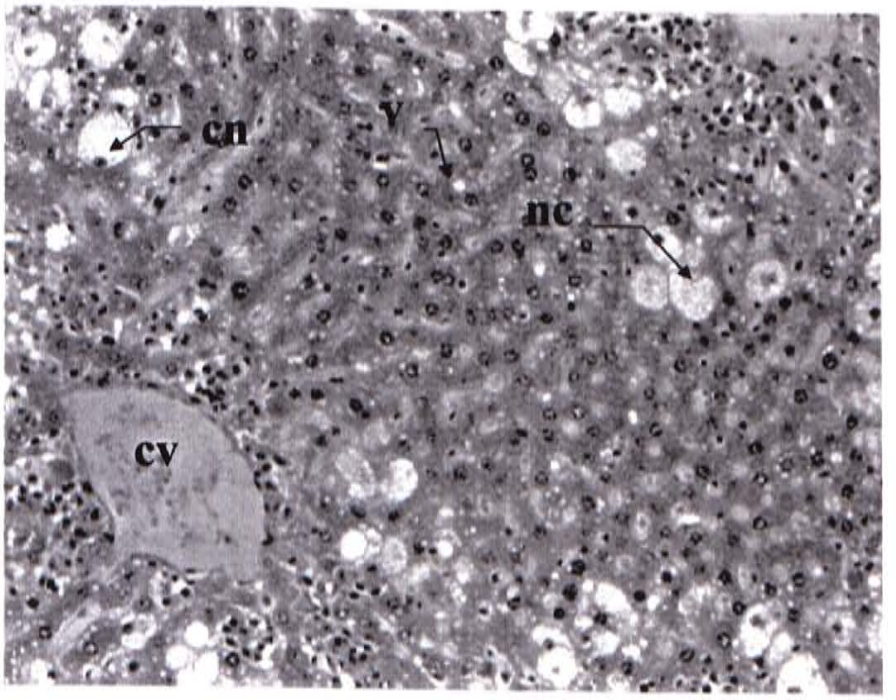
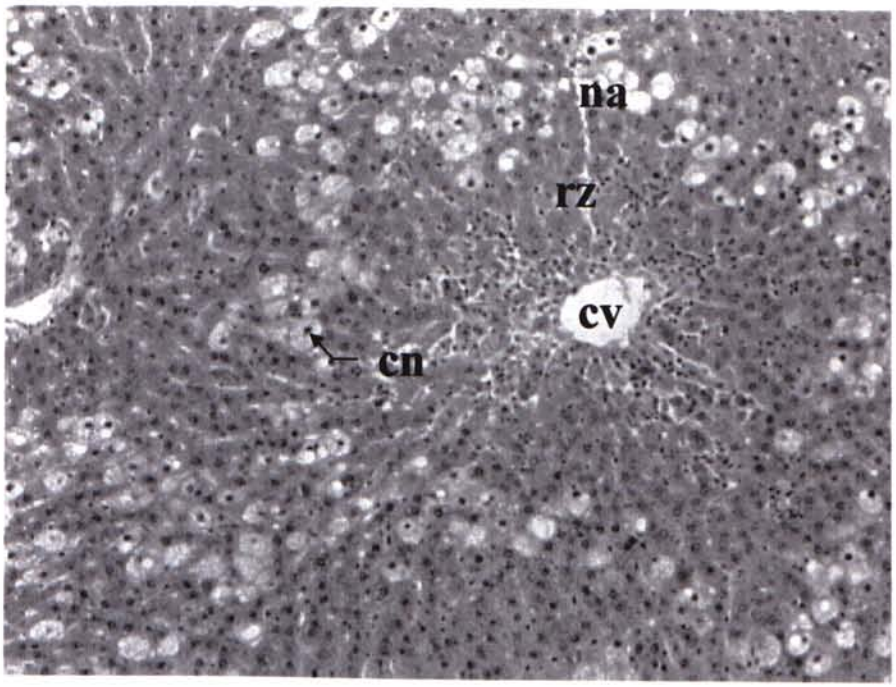

cn
v
nc
cv

Fig. 4.96 Effect of the seaweed extract (S#4: *S. siliquastrum*, 60 mg/ml saline) on the liver of CCl_4-treated rat showing necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. Medium regeneration zone can hardly be seen. cv, central vein; cn, condensed nucleus; nc, necrotic cell; rz, regeneration zone.
(95 ×, H & E)

Fig. 4.97 Effect of the seaweed extract (S#4: *S. siliquastrum*, 60 mg/ml saline) on the liver of CCl_4-treated rat showing necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cv, central vein; cn, condensed nucleus; mf, mitotic figure; nc, necrotic cell; p, portal triad; rz, regeneration zone.
(192 ×, H & E)


na
rz
cv
cn

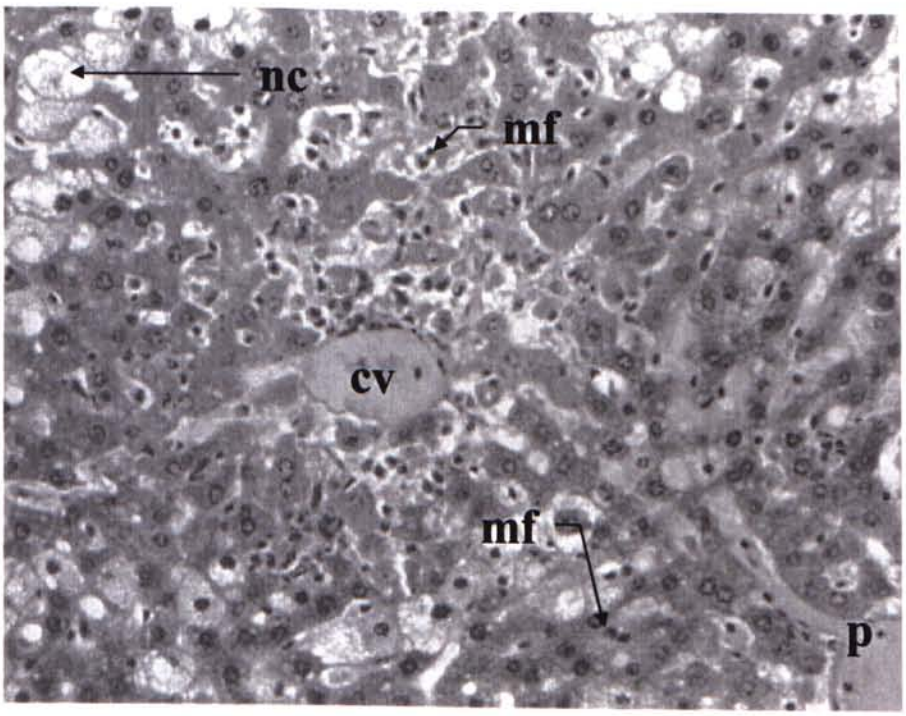
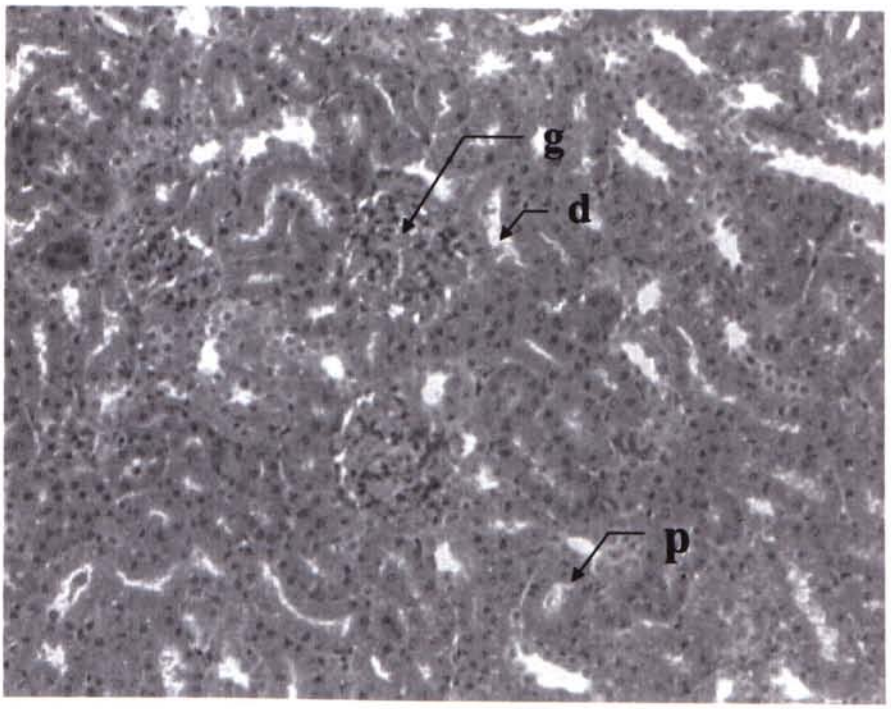

nc
mf
cv
mf
p

Fig. 4.98 Micrograph of the liver of rat from the vehicle-saline curative group (renal cortex) showing normal kidney cells. d, distal tubules; g, glomerulus; p, proximal tubules
(155x, H & E)

Fig. 4.99 Micrograph of the liver of rat from the vehicle-saline curative group (renal cortex) showing normal kidney cells showing normal kidney cells. bb, brush border; d, distal tubules; g, glomerulus; p, proximal tubules
(307x, H & E)


g
d
p

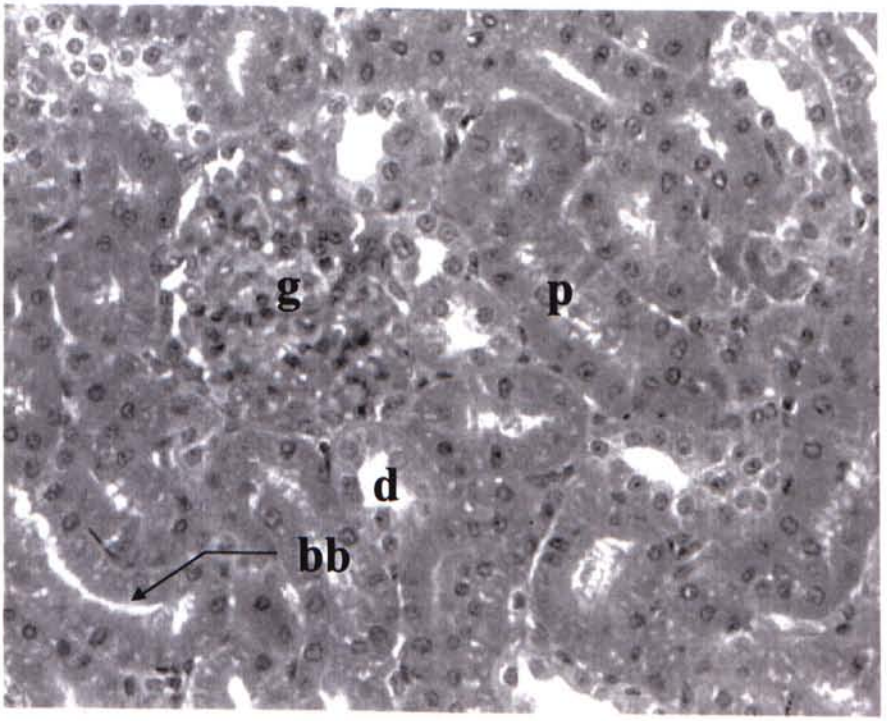
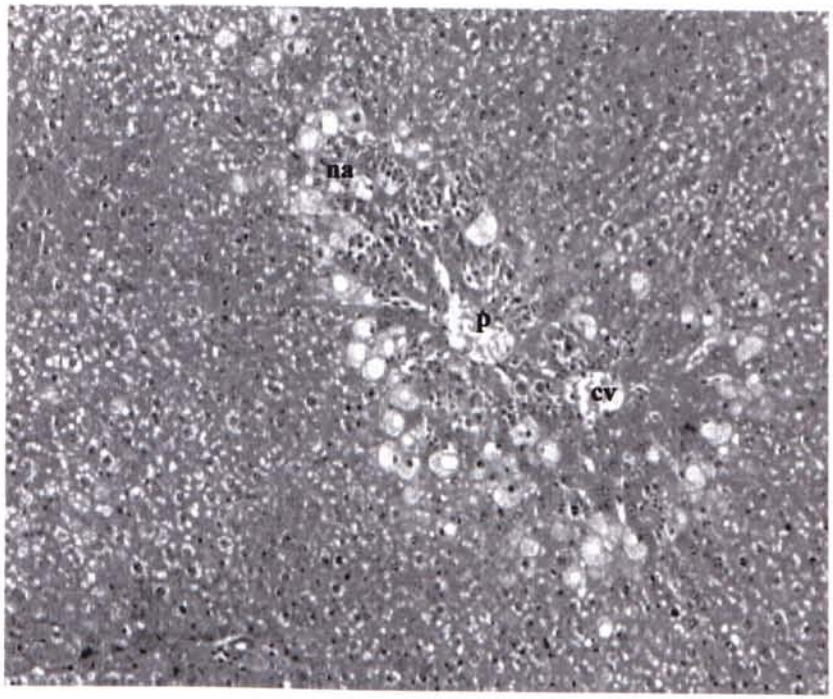

g
p
d
bb

Fig. 4.100 Micrograph of the liver of CCl_4-treated rat from toxin control preventive group showing extensive necrosis of hepatocytes around the central vein region as compared with the vehicle-saline curative group. cv, central vein; na, necrotic area; p, portal triad.
(97 ×, H & E)

Fig. 4.101 Micrograph of the liver of CCl_4-treated rat from toxin control curative group showing showing extensive necrosis of hepatocytes around the central vein region as compared with the vehicle-saline curative group. cn, condensed nucleus; cv, central vein; na, necrotic area; p, portal triad; sc, swellen cell; v, vacuolation
(192 ×, H & E)


na
p
cv

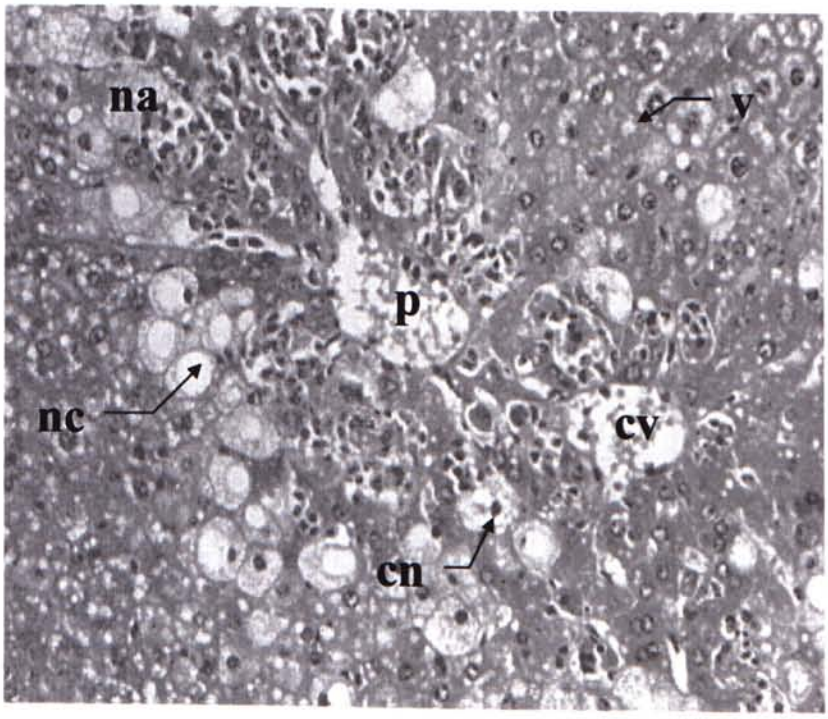
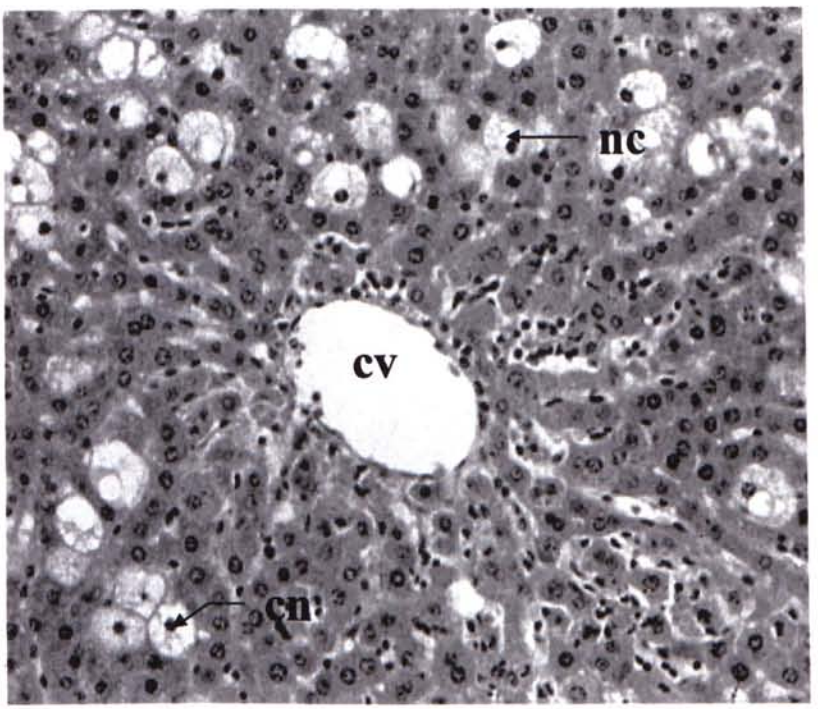

na
v
p
nc
cv
cn

Fig. 4.102 Effect of the seaweed extract (S#2: *S. henslowianum*, 60 mg/ml saline), preventive, on the liver of CCl_4-treated rat showing extensive necrosis of hepatocytes around the central vein region as compared with the toxin control preventive group. cn, condensed nucleus; cv, central vein; nc, necrotic cell.
(194 ×, H & E)

Fig. 4.103 Effect of the seaweed extract (S#4: *S. siliquastrum*, 60 mg/ml saline) on the liver of CCl_4-treated rat showing extensive necrosis of hepatocytes around the central vein region as compared with the toxin control preventive group. cv, central vein; cn, condensed nucleus; nc, necrotic cell; v, vacuolization.
(196 ×, H & E)


nc
cv
cn

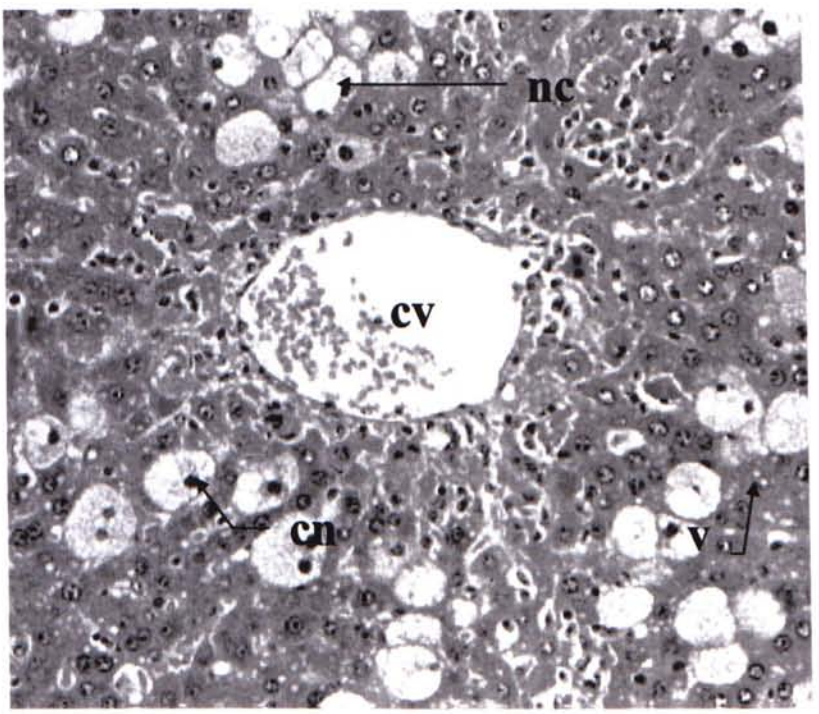
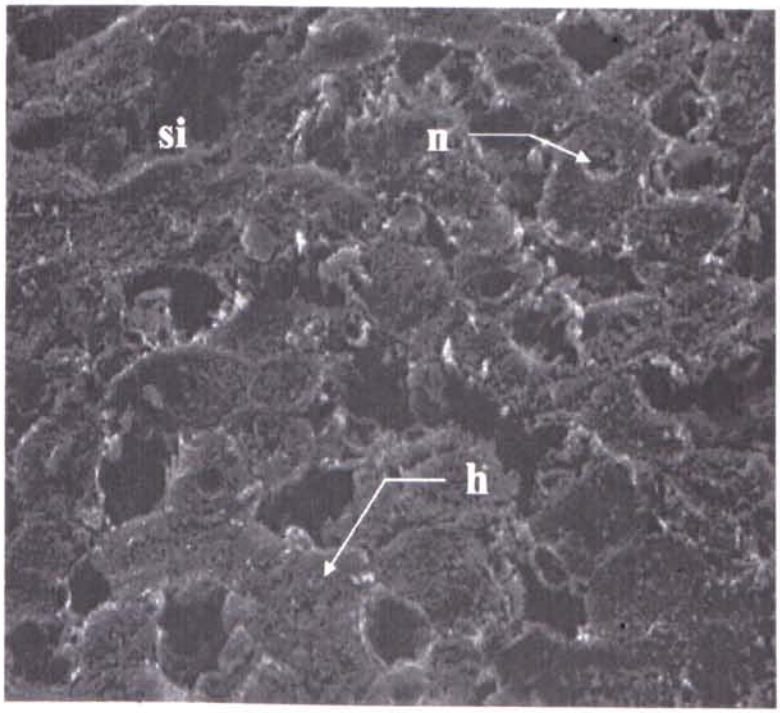

nc
cv
cn
v

Fig. 4.104 Scanning electron micrograph of the liver of rat from the vehicle-saline curative group showing normal hepatocytes cords linings. h, hepatocyte; n, normal nuclues; si, sinusoid.
(1 200 ×)

Fig. 4.105 Scanning electron micrograph of the liver of CCl_4-treated rat from toxin control curative group showing extensive necrosis of hepatocytes around the central vein region and the clear vacuolization as compared with the vehicle-saline curative group. cv, central vein; na, necrotic area; nc, necrotic cell; gv, groups of vacuolization
(620 ×)


si
n
h

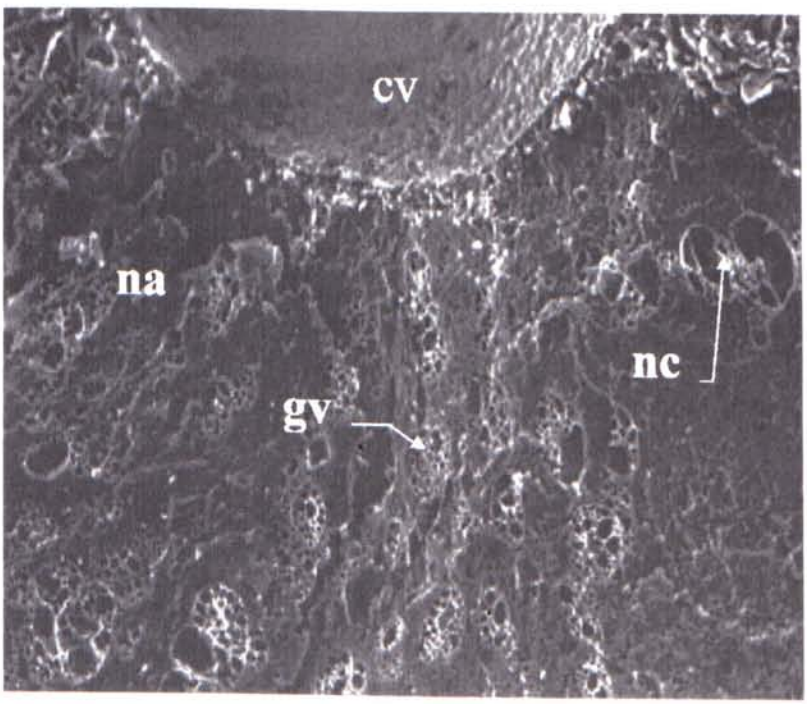
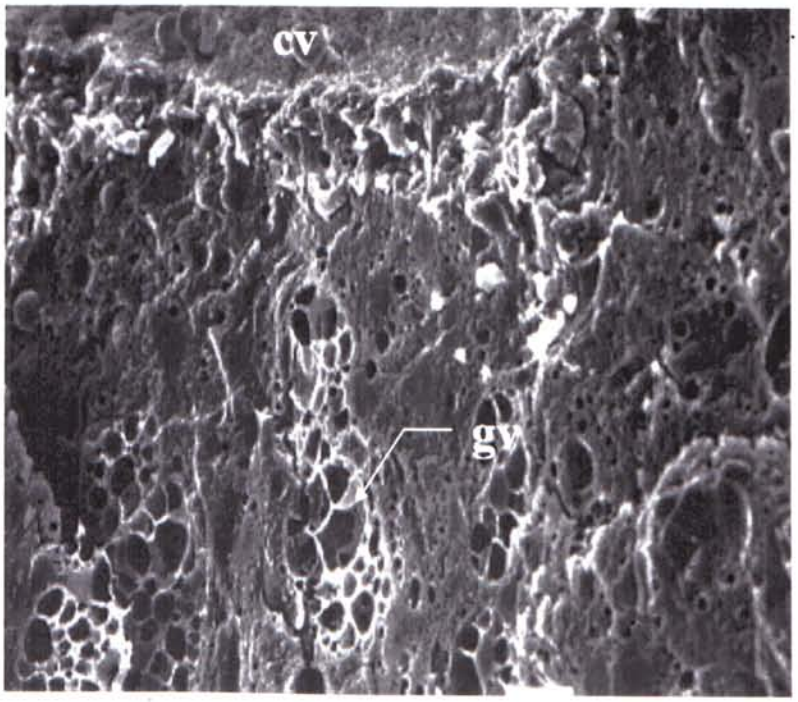

cv
na
nc
gv

Fig. 4.106 Scanning electron micrograph of the liver of CCl_4-treated rat from toxin control curative group showing extensive necrosis of hepatocytes around the central vein region and the clear of vacuolization as compared with the vehicle-saline curative group. cv, central vein; gv, groups of vacuolization
(1 800 ×)

Fig. 4.107 Scanning electron micrograph of the liver of CCl_4-treated rat (duplicated) from toxin control curative group showing extensive necrosis of hepatocytes around the central vein region and the clear of vacuolization as compared with the vehicle-saline curative group. cv, central vein; gv, groups of vacuolization; nc, necrotic cell; v, vacuolization
(1 800 ×)


cv
gv

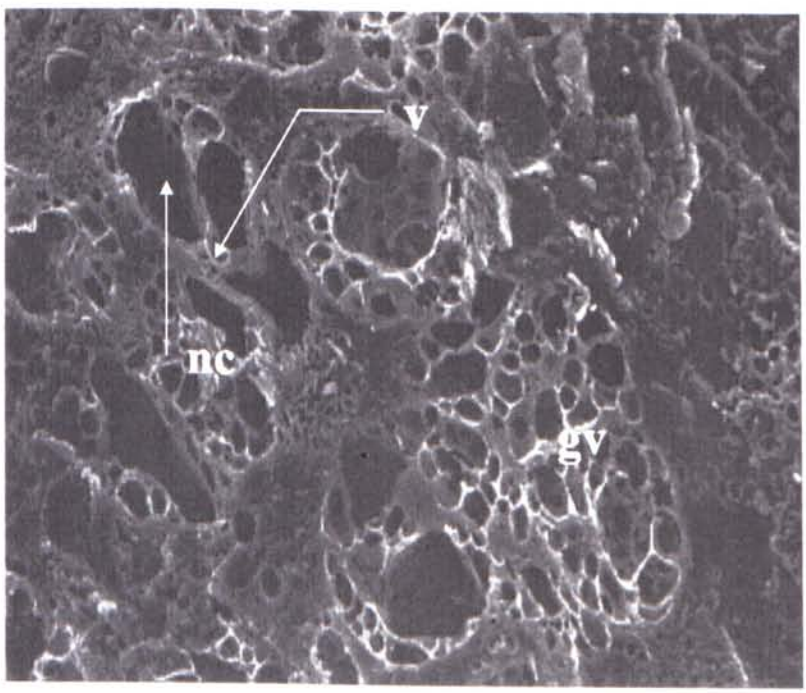
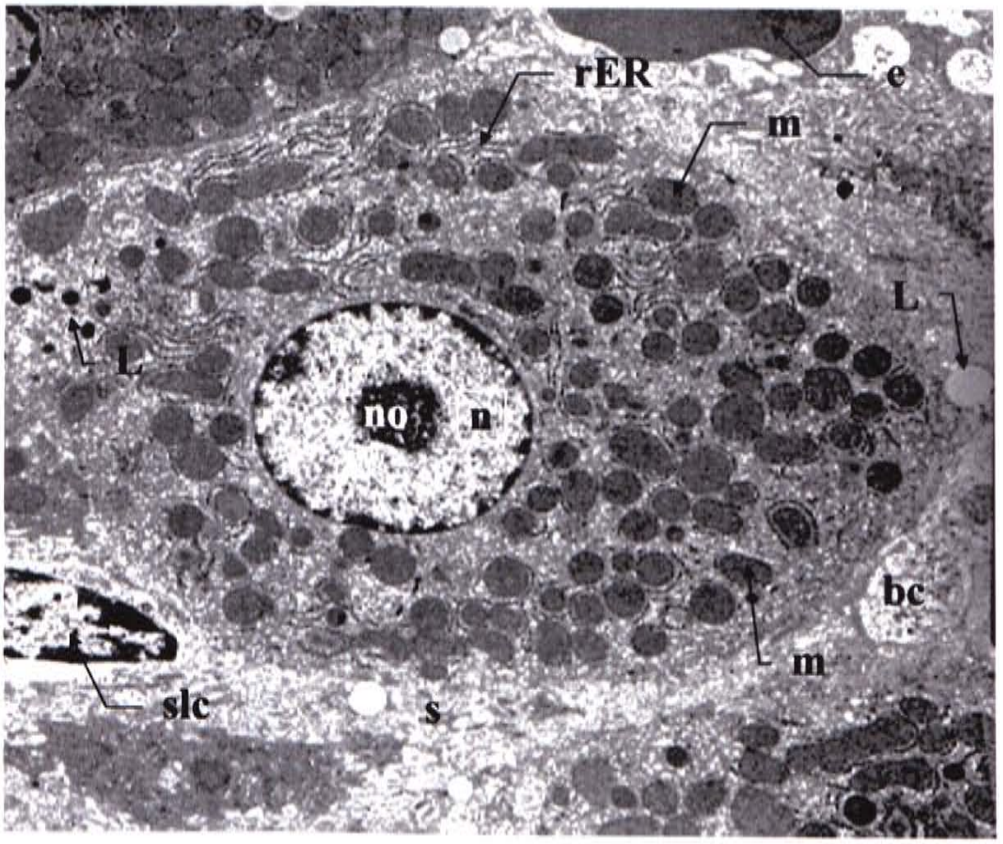

v
nc
gv

Fig. 4.108 Transmission electron micrograph of the rat from the vehicle-saline curative group showing normal hepatocyte with clear shown of organelles inside. bc, bile canaliculi; e, erythrocyte; L, lipid droplet; Ly, lysosome; m, mitochondria; n, normal nucleus; no, nucleolus; rER, rough endoplasmic reticulum; si, sinusoid; slc, sinusoid lining cell. (5 700 ×)

Fig. 4.109 Transmission electron micrograph of the rat from the vehicle-saline curative group showing normal hepatocyte with clear shown of organelles inside. bc, bile canaliculi; L, lipid droplet; Ly, lysosome; m, mitochondria; n, normal nucleus; rER, rough endoplasmic reticulum. (8 800 ×)


rER
e
m
L
L
no
n
bc
m
slc
s

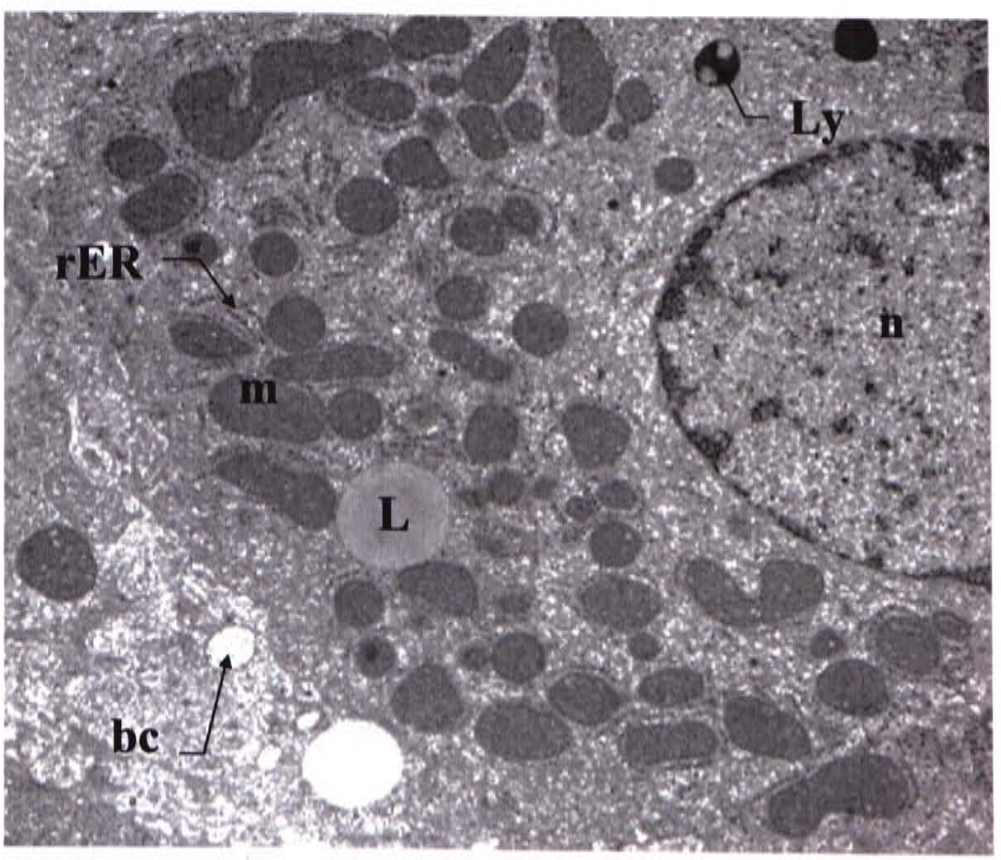
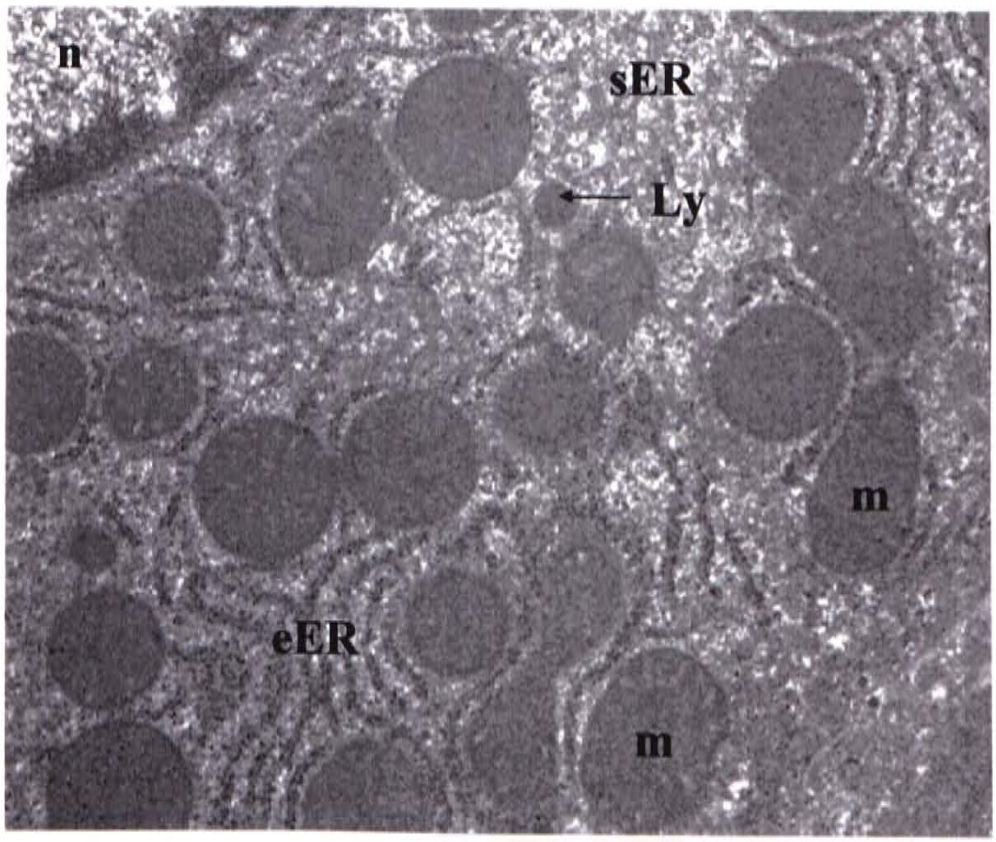

Ly
rER
n
m
L
bc

Fig. 4.110 Transmission electron micrograph of the liver of rat from the vehicle-saline curative group showing normal hepatocyte with clear shown of organelles inside. Ly, lysosome; m, mitochondria; n, normal nucleus; rER, rough endoplasmic reticulum; sER, smooth endoplasmic reticulum.
(20 000 x)

Fig. 4.111 Transmission electron micrograph of the liver of rat from the vehicle-saline curative group showing normal hepatocyte with clear shown of organelles inside. m, mitochondria; r, ribosome; rER, rough endoplasmic reticulum.
(44 000 x)


n
sER
Ly
m
eER
m

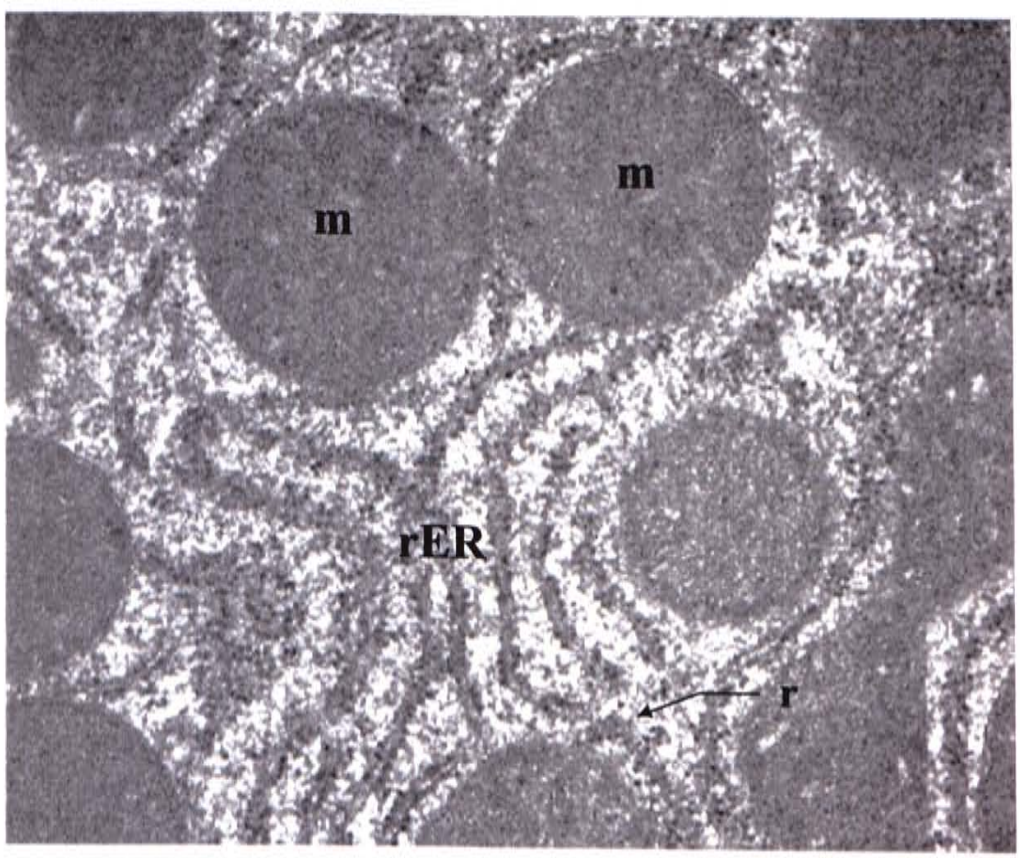
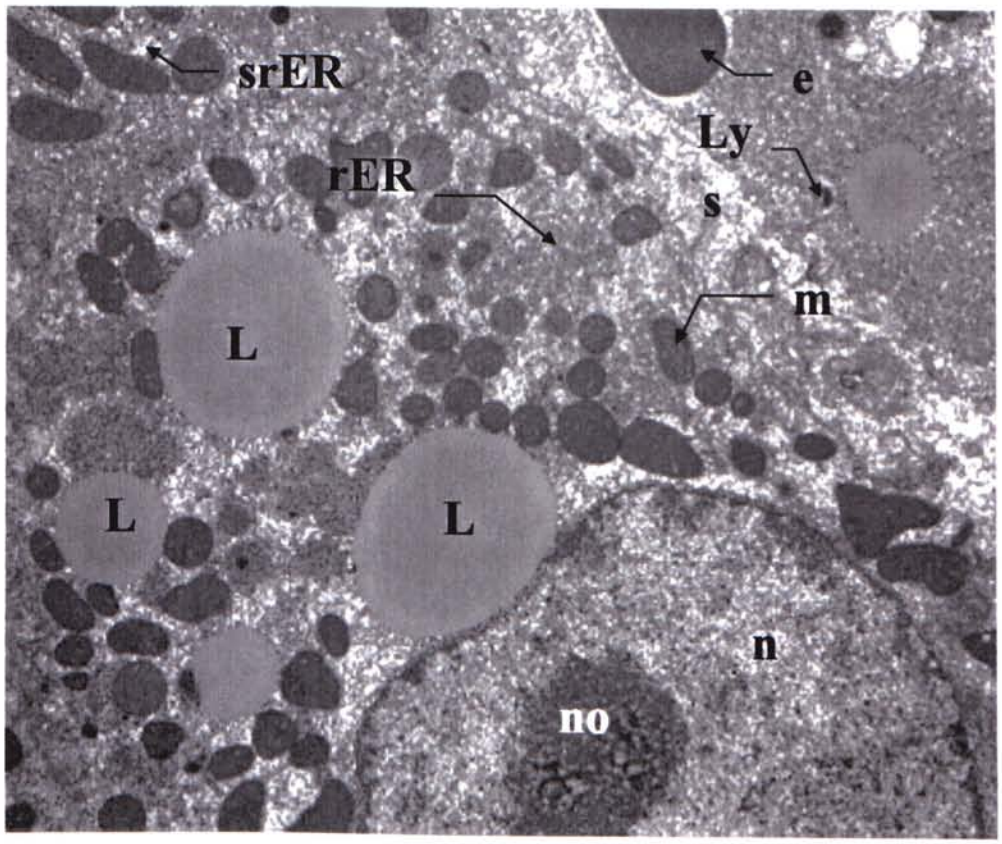

m
m
rER
r

Fig. 4.112 Transmission electron micrograph of the liver of CCl_4-treated rat from the toxin control curative group: showing early stage of injury in certain area. e, erythrocyte; L, lipid droplet; Ly, lysosome; m, mitochondria; n, normal nucleus; no, nucleolus; rER, rough endoplasmic reticulum; si, sinusoid; srER, swollen rough endoplasmic reticulum.
(7 200 ×)

Fig. 4.113 Transmission electron micrograph of the liver of CCl_4-treated rat from the toxin control curative group showing early stage of injury in hepatocyte. e, erythrocyte; L, lipid droplet; sm, swollen mitochondria; an, abnormal nucleus; si, sinusoid; v, vacuolization.
(5 700 ×)


srER
e
Ly
rER
s
m
L
L
L
n
no

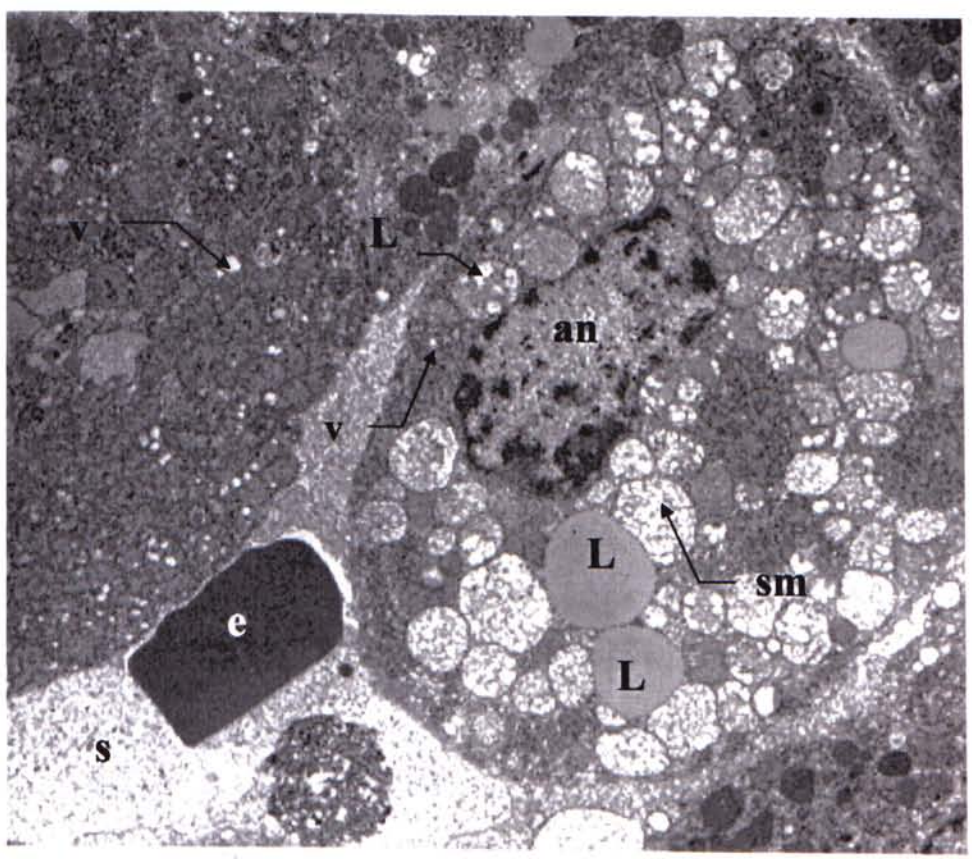
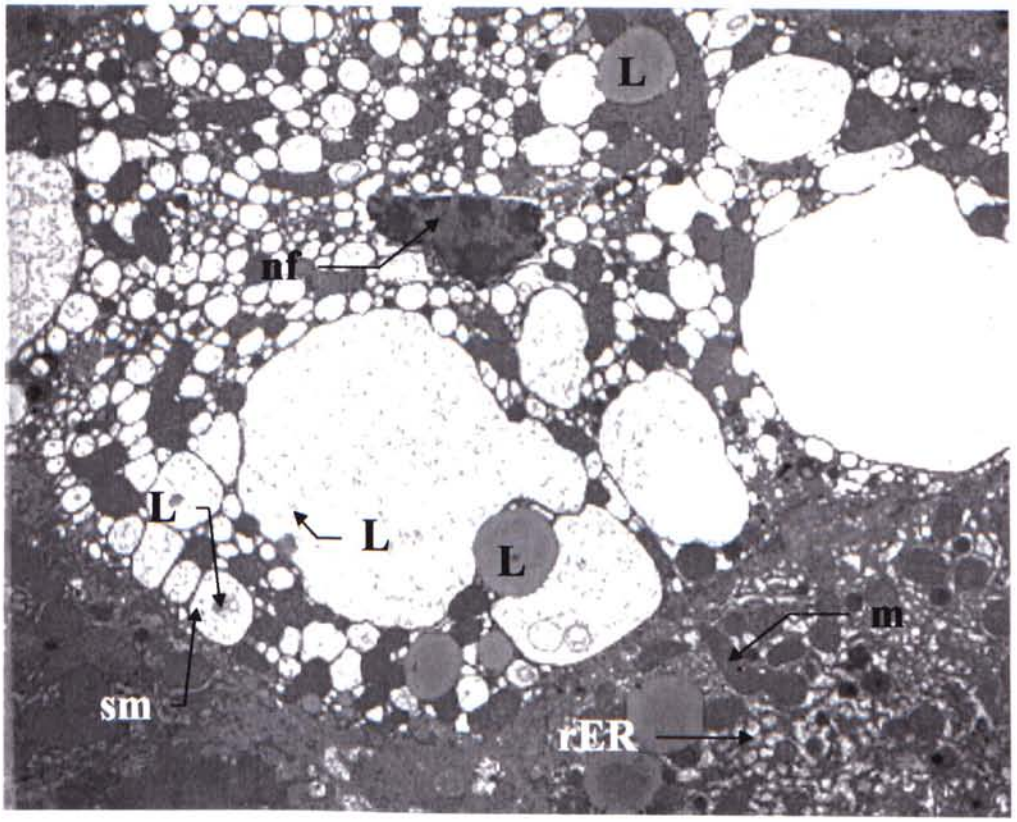

v
L
an
v
L
sm
e
L
s

Fig. 4.114 Transmission electron micrograph of the liver of CCl_4-treated rat from the toxin control curative group showing final stage of injury in hepatocyte, necrotic cell. L, lipid droplet; m, mitochondria; sm, swollen mitochondria; nf, fragment of nucleus; v, vacuolization.
(5 700 x)

Fig. 4.115 Transmission electron micrograph of the liver of CCl_4-treated rat from the toxin control curative group showing early stage of injury of organelles inside hepatocyte. m, mitochondria; srER, swollen rough endoplasmic reticulum.
(36 000 x)


L
nf
L
L
L
m
sm
rER

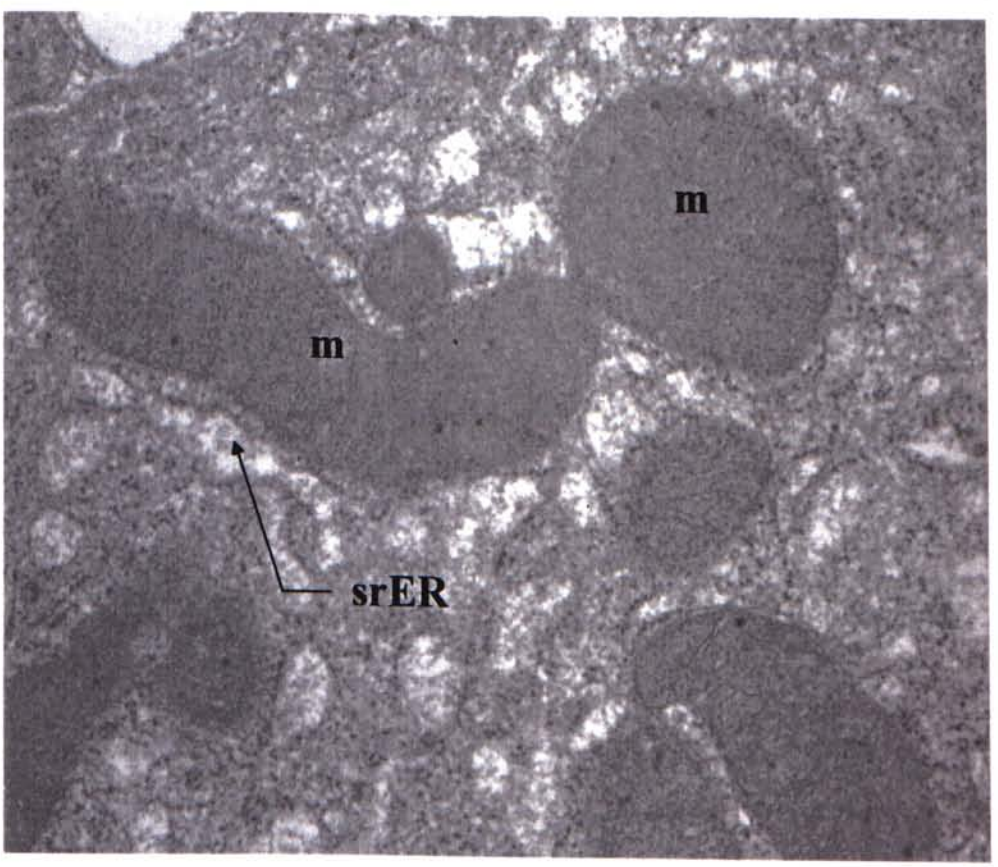

m
m
srER

Fig. 4.116 Transmission electron micrograph of the liver of CCl_4-treated rat from the toxin control curative group showing early stage of injury of organelles inside hepatocyte. cr, cristae; L, lipid droplet; sm, swollen mitochondria; srER, swollen rough endoplasmic reticulum.
(36 000 ×)

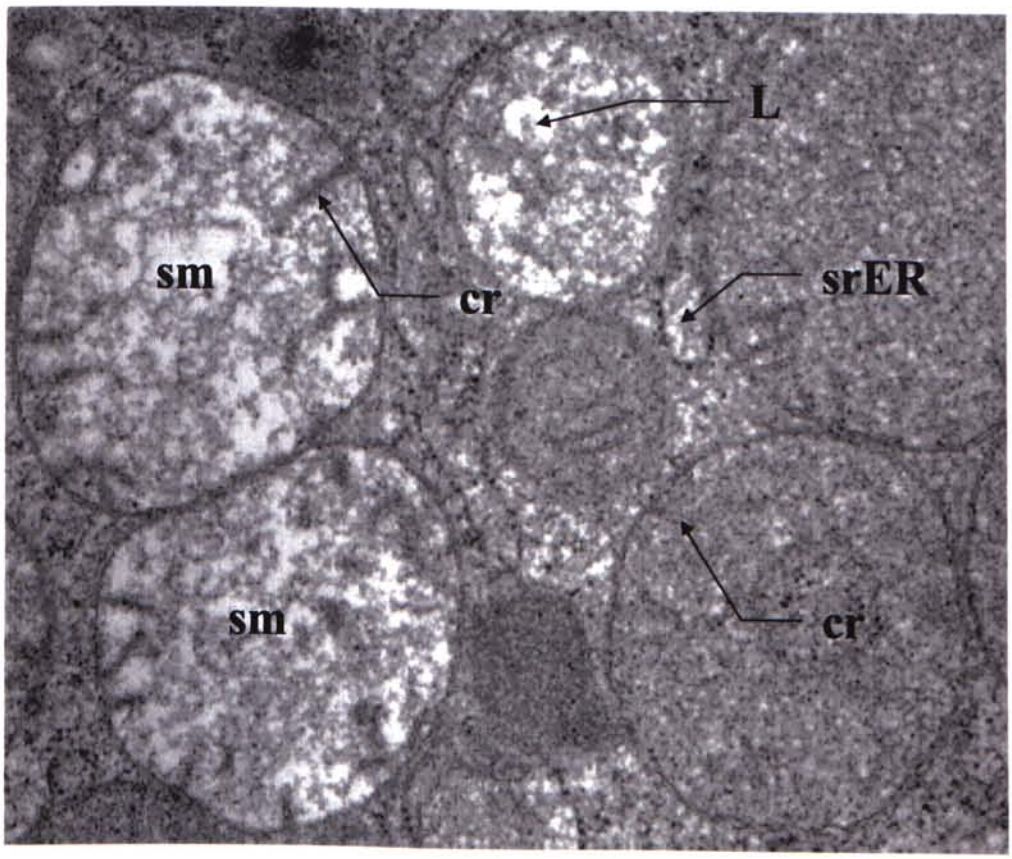
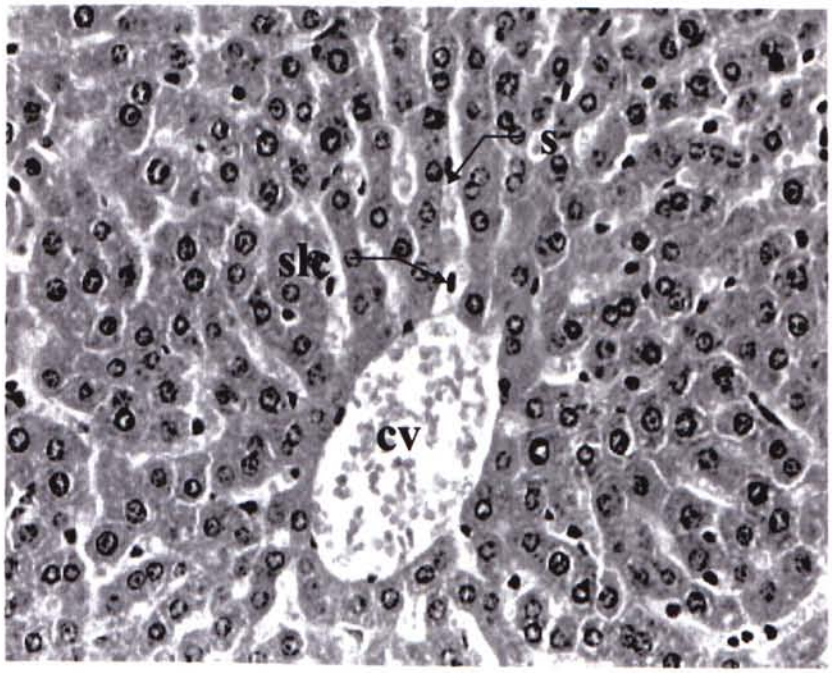

L
sm
cr
srER
sm
cr

Fig. 4.117 Micrograph of the liver of rat from vehicle-saline curative group in i.p. showing normal hepatocytes around the central vein region. cv, central vein; si, sinusoid; slc, sinusoid lining cell.
(227 x, H & E)

Fig. 4.118 Micrograph of the liver of 20% TCE-treated (i.p.) rat from effective dose of curative group showing no necrosis of hepatocytes around the central vein region as compared with the control group (Fig. 4.117). cv, central vein; si, sinusoid; slc, sinusoid lining cell.
(232 x, H & E)


s
slc
cv

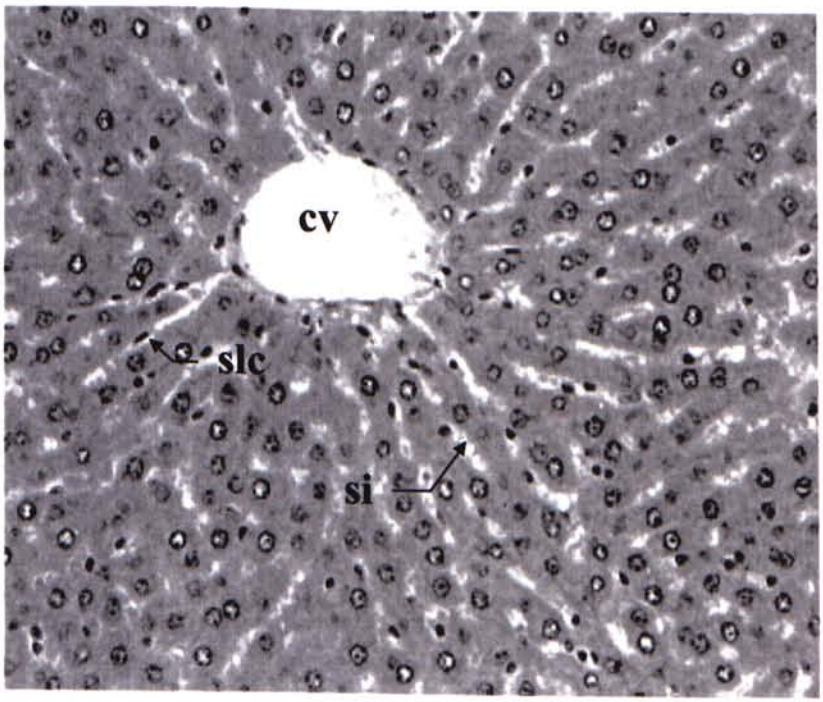

cv
slc
si

Fig. 4.119 Micrograph of the renal cortex of 20% TCE-treated (i.p.) rat from effective dose of curative group showing normal kidney cell. bb, brush border; d, distal tubules; g, glomerulus; p, proximal tubules. (188 ×, H & E)

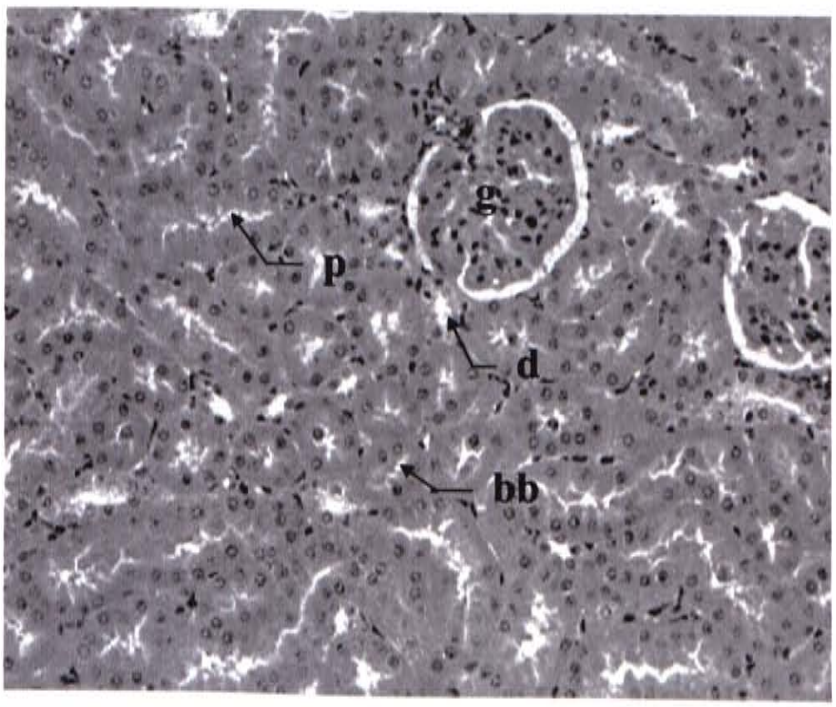
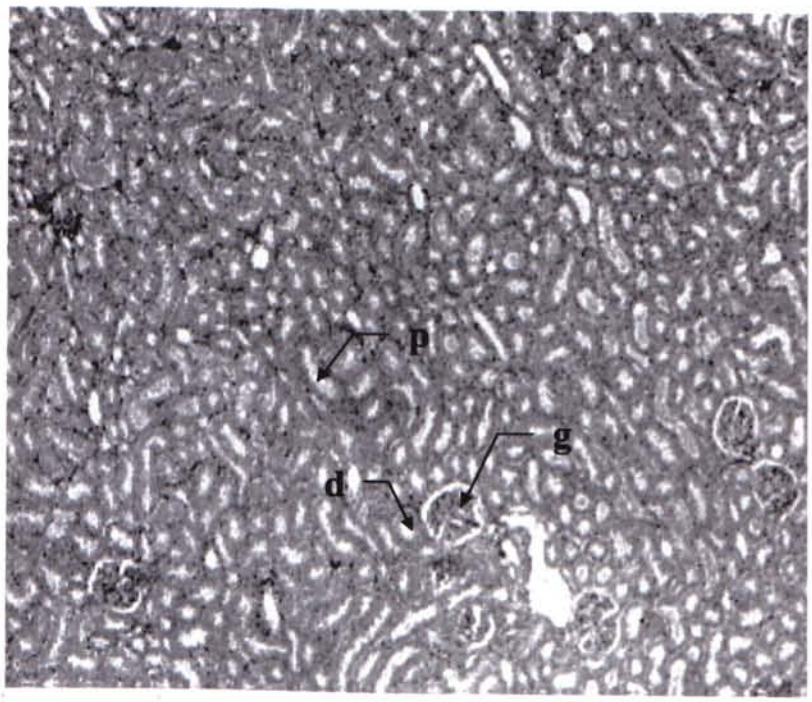

g
p
d
bb

Fig. 4.120 Micrograph of the renal cortex of 30% TCE-treated (i.p.) rat from the TCE curative group showing abnormal kidney cell (dilated tubules). d, distal tubules; g, glomerulus; p, proximal tubules.
(60 ×, H & E)

Fig. 4.121 Micrograph of the renal cortex of 30% TCE-treated (i.p.) rat from the TCE curative group showing abnormal kidney cell (dilated tubules). bb, brush border; cd, cell debris, d, distal tubules; g, glomerulus; p, proximal tubules.
(153 ×, H & E)


p
g
d

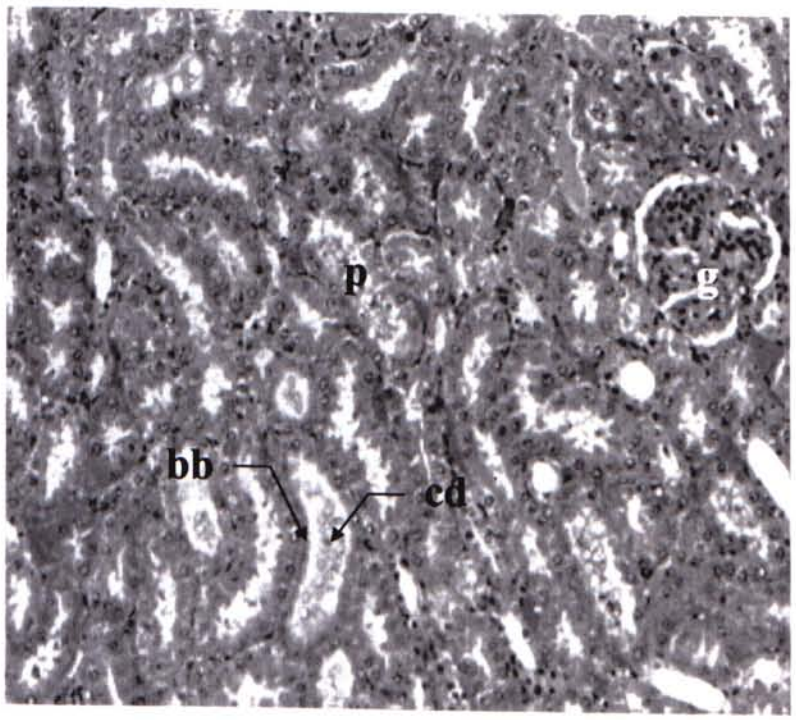
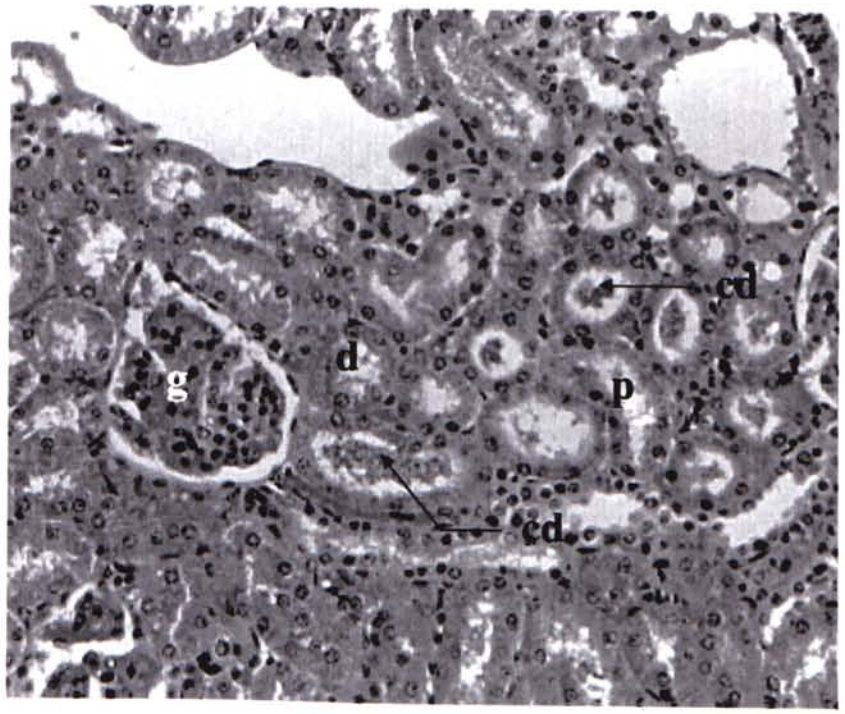

p
g
bb
cd

Fig. 4.122 Micrograph of the renal cortex of 30% TCE-treated (i.p.) rat from the TCE curative group showing abnormal kidney cell (dilated tubules). cd, cell debris, d, distal tubules; g, glomerulus; p, proximal tubules. (188 ×, H & E)

Fig. 4.123 Micrograph of the proximal tubules of 30% TCE-treated (i.p.) rat from the TCE curative group showing dilated proximal tubules with cell debris inside. bb, brush border; cd, cell debris; l, lumen. (593 ×, H & E)


cd
g
d
p
cd

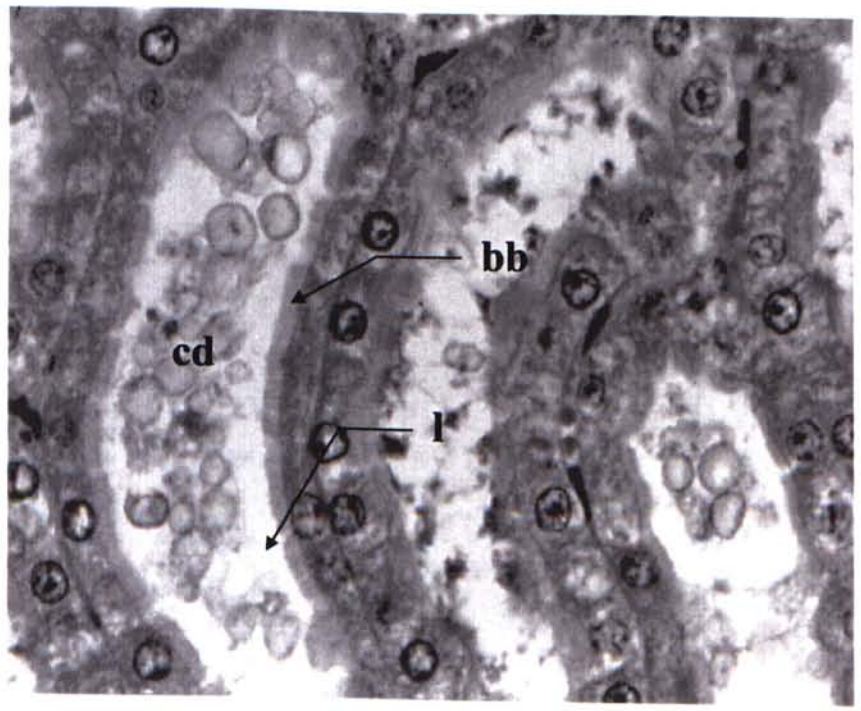
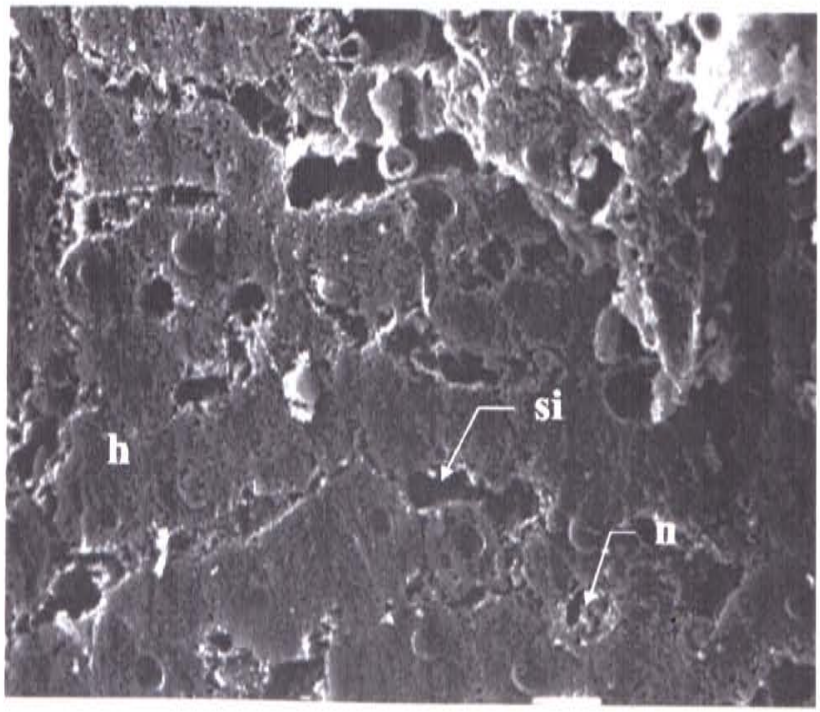

l
bb
cd
l

Fig. 4.124 Scanning electron micrograph of liver of 20% TCE-treated (i.p.) rat from effective dose of curative group showing normal hepatocyte cords linings. h, hepatocyte; n, normal nuclues; si, sinusoid
(1 800 ×)

Fig. 4.125 Scanning electron micrograph of liver of 20% TCE-treated (i.p.) rat (duplicated) from effective dose of curative group showing normal hepatocytes cords linings. h, hepatocyte; n, normal nuclues; si, sinusoid
(2 600 ×)


si
h
n

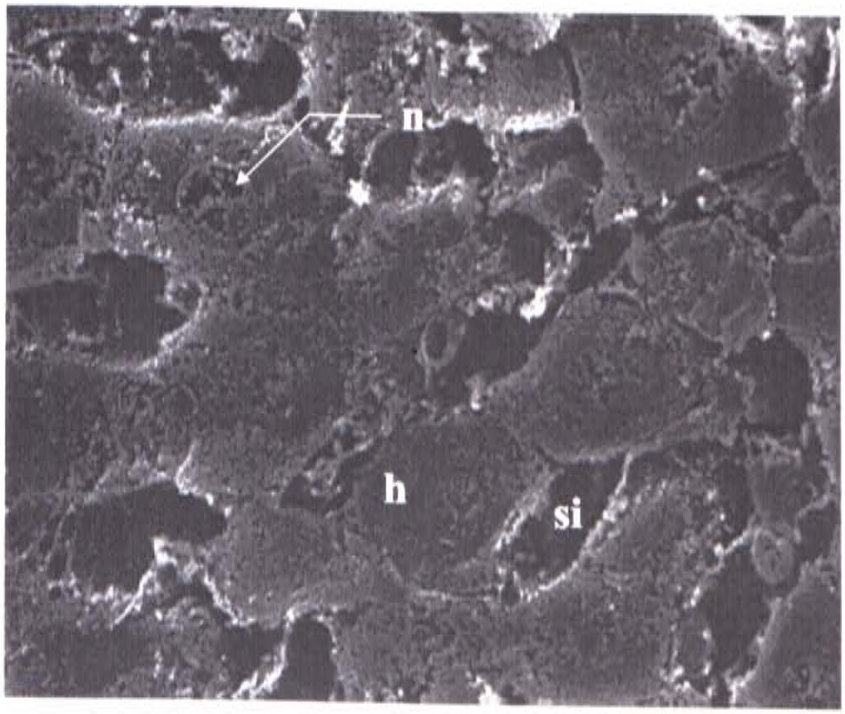
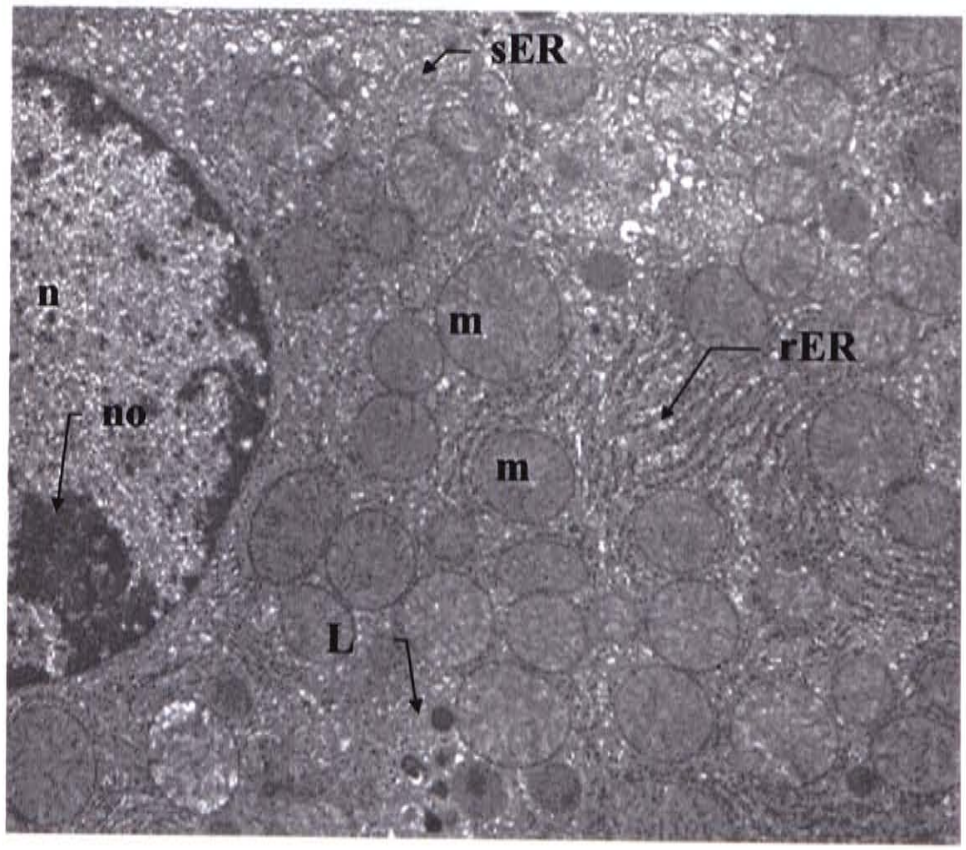

n
h
si

Fig. 4.126 Transmission electron micrograph of the liver of 20% TCE-treated (i.p.) rat from effective dose of curative group showing normal hepatocyte with clear shown of organelles inside (no signs of abnormal). Ly, lysosome; m, mitochondria; n, normal nucleus; no, nucleolus; rER, rough endoplasmic reticulum; sER, smooth endoplasmic reticulum.
(1 2000 ×)

Fig. 4.127 Transmission electron micrograph of liver of 20% TCE-treated (i.p.) rat from effective dose of curative group showing hepatocyte with many large vacuoles with lipid inside. m, mitochondria; n, normal nucleus; rER, rough endoplasmic reticulum; vo, vacoule
(1 5000 ×)


sER
n
m
rER
no
m
L

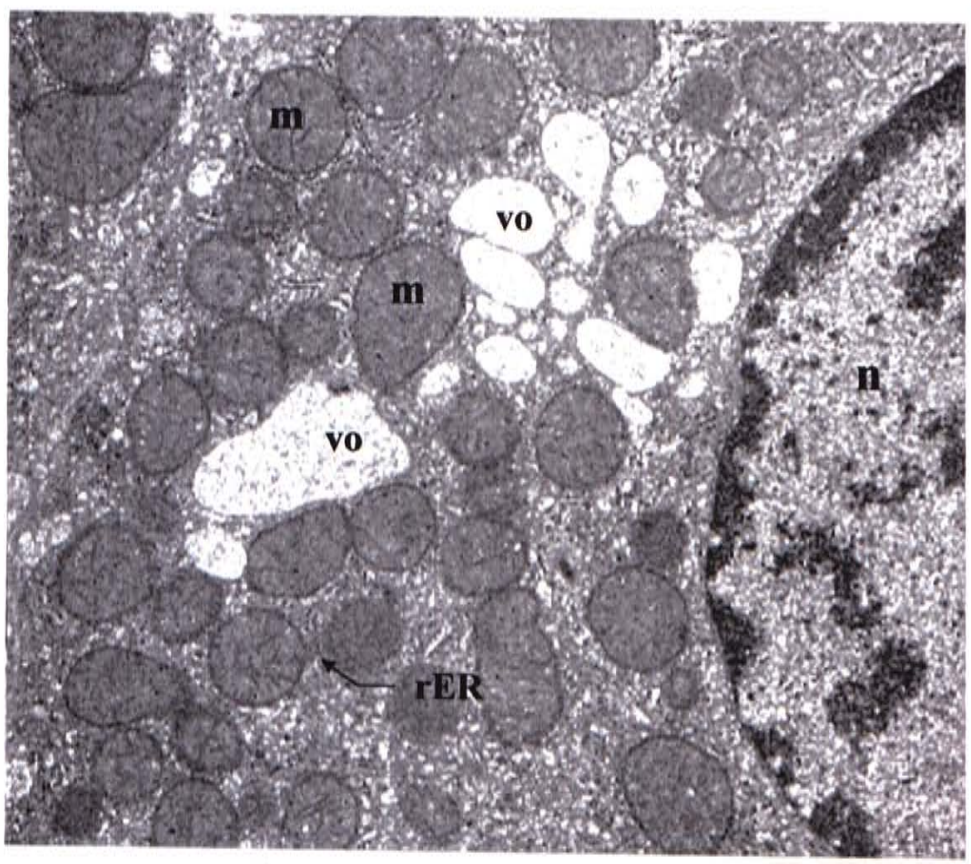
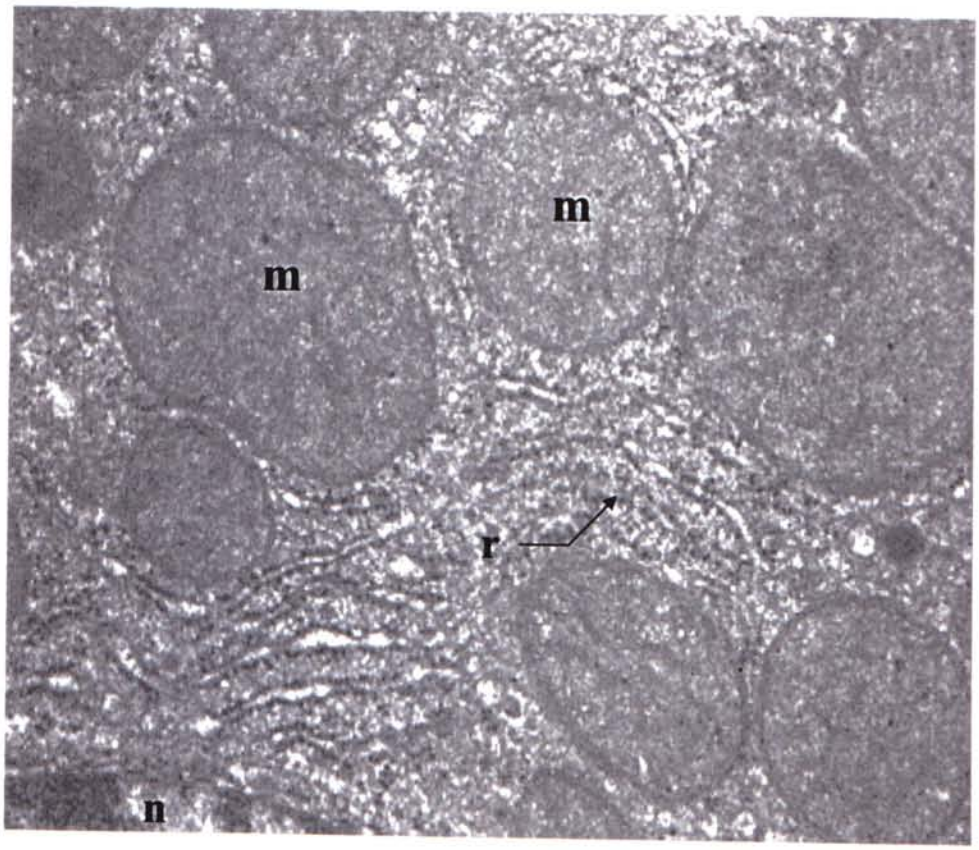

m
vo
m
n
vo
rER

Fig. 4.128 Transmission electron micrograph of the liver of 20% TCE-treated (i.p.) rat from effective dose of curative group showing mitochondria and slightly swollen rough endoplasmic reticulum. m, mitochondria; n, normal nucleus; r, ribosome; srER, swollen rough endoplasmic reticulum.
(3 6000 ×)

Fig. 4.129 Transmission electron micrograph of the liver of 20% TCE-treated (i.p.) rat (duplicated) from effective dose of curative group showing mitochondria and slightly swollen rough endoplasmic reticulum. cr, cristae; Ly, lysosome; m, mitochondria; r, ribosome; srER, swollen rough endoplasmic reticulum.
(3 6000 ×)


m
m
r
n

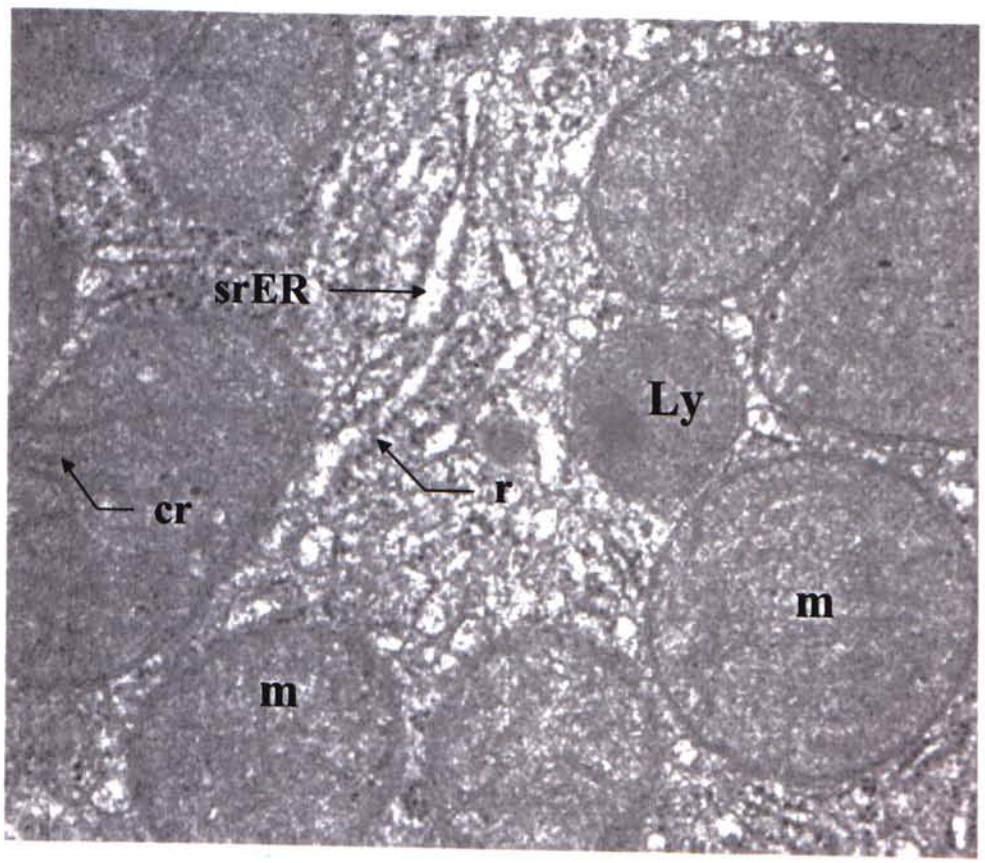
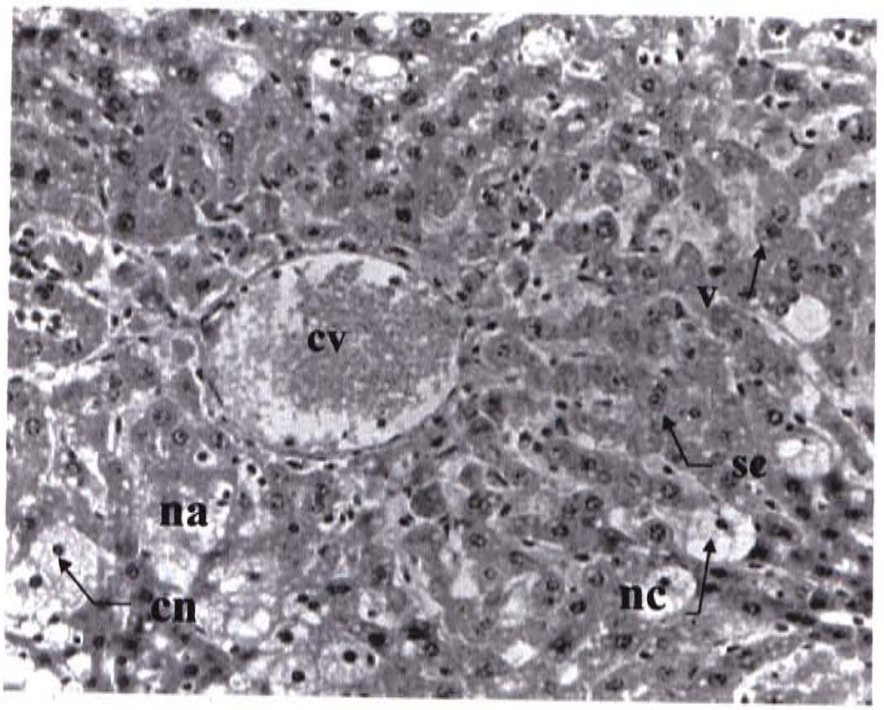

srER
Ly
r
cr
m
m

Fig. 4.130 Effect of the DMSO 25% on the liver of CCl_4-treated rat showing massive necrosis of hepatocytes around the central vein region as compared with the toxin control curative group. cv, central vein; cn, condensed nucleus; na, necrotic area; nc, necrotic cell; v, vacuolization; sc, swollen cell.
(188 x, H & E)

Fig. 4.131 Effect of the DMSO 75% on the liver of CCl_4-treated rat showing few necrosis of hepatocytes with large range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; na, necrotic area; rz, regeneration zone.
(96 x, H & E)


cv
v
se
na
cn
nc

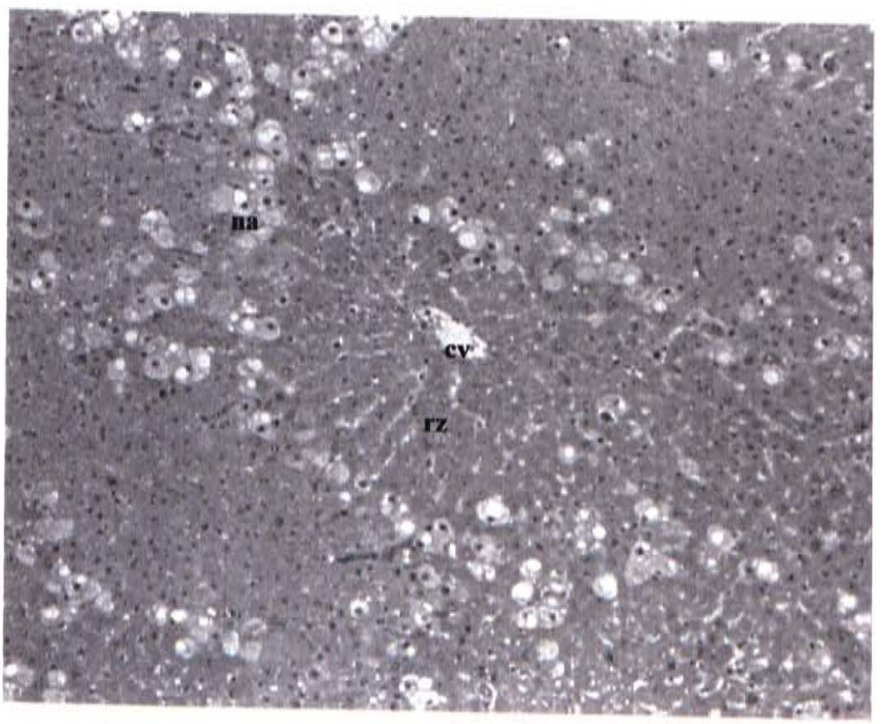
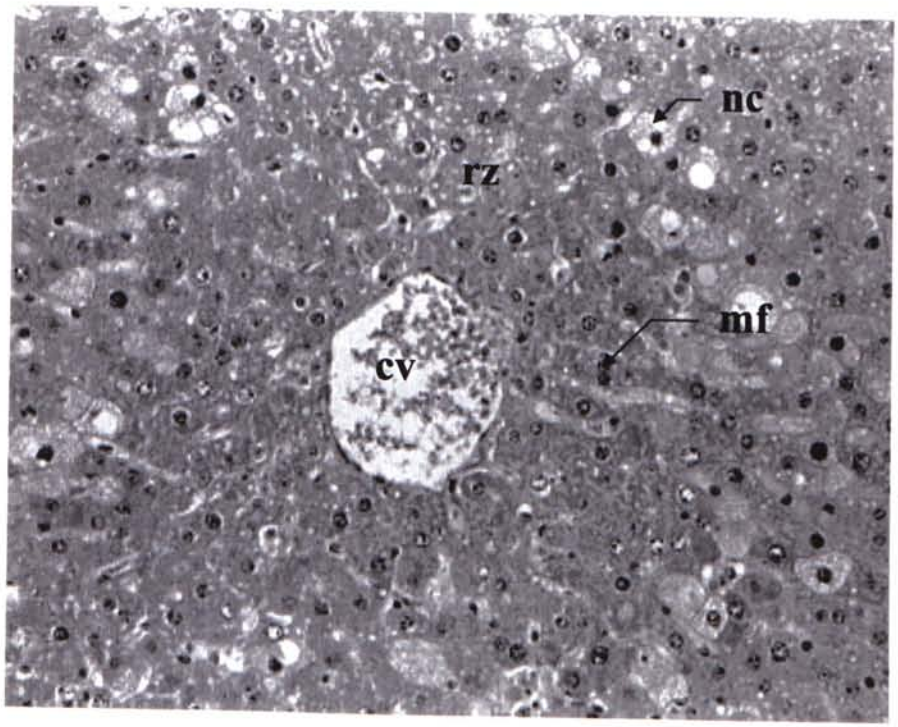

na
cv
rz

Fig. 4.132 Effect of the DMSO 75% on the liver of CCl_4-treated rat (duplicated) showing few necrosis of hepatocytes with large range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; cn, condensed nucleus; mf, mitotic figure; na, necrotic area; nc, necrotic cell; v, vacuolization; sc, swollen cell

(194 ×, H & E)

Fig. 4.133 Effect of the NAC (15 mg/ml saline) on the liver of CCl_4-treated rat showing massive necrosis of hepatocytes with small range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; na, necrotic area; rz, regeneration zone.

(61 ×, H & E)


nc
rz
mf
cv

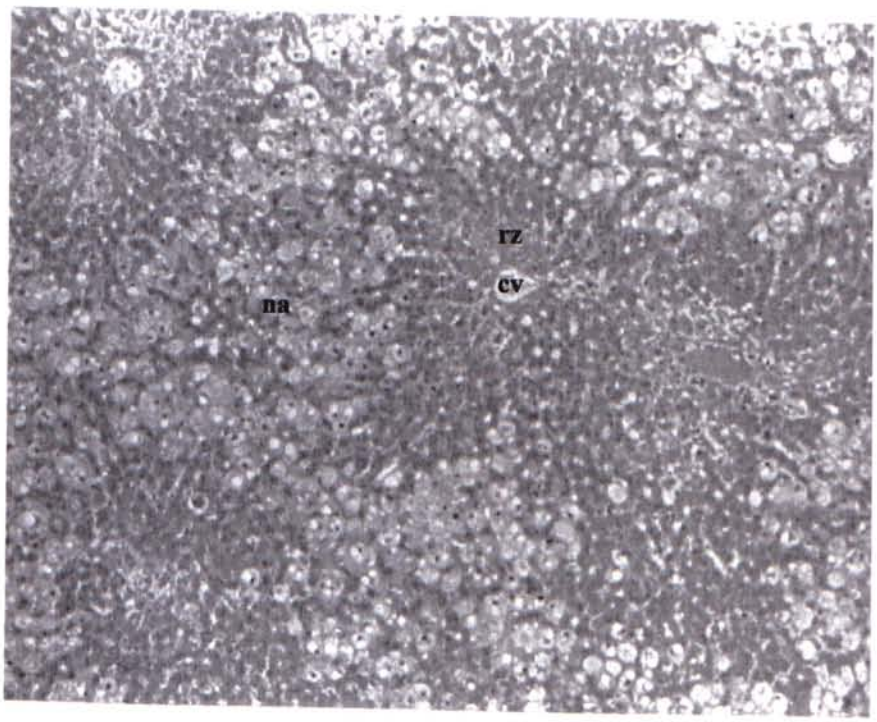
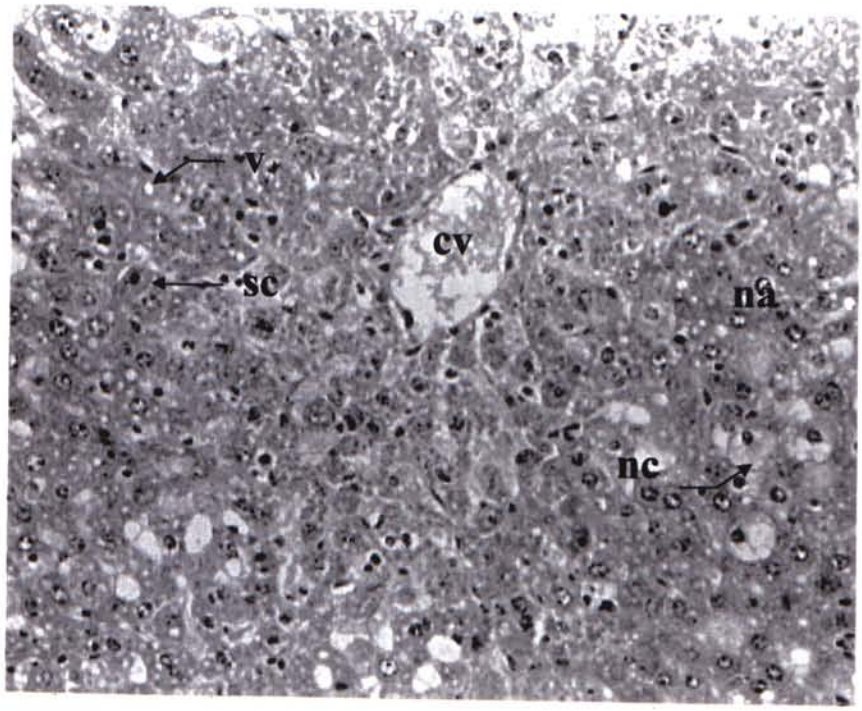

rz
cv
na

Fig. 4.134 Effect of the NAC (15 mg/ml saline) on the liver of CCl_4-treated rat showing massive necrosis of hepatocytes with small range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; na, necrotic area; sc, swollen cell; v, vacuolization.
(190 ×, H & E)

Fig. 4.135 Effect of the NAC (15 mg/ml saline) on the liver of CCl_4-treated rat (duplicated) showing massive necrosis of hepatocytes with small range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; cn, condensed nucleus; na, necrotic area; nc, necrotic cell; sc, swollen cell; v, vacuolization.
(297 ×, H & E)


v
cv
sc
na
nc

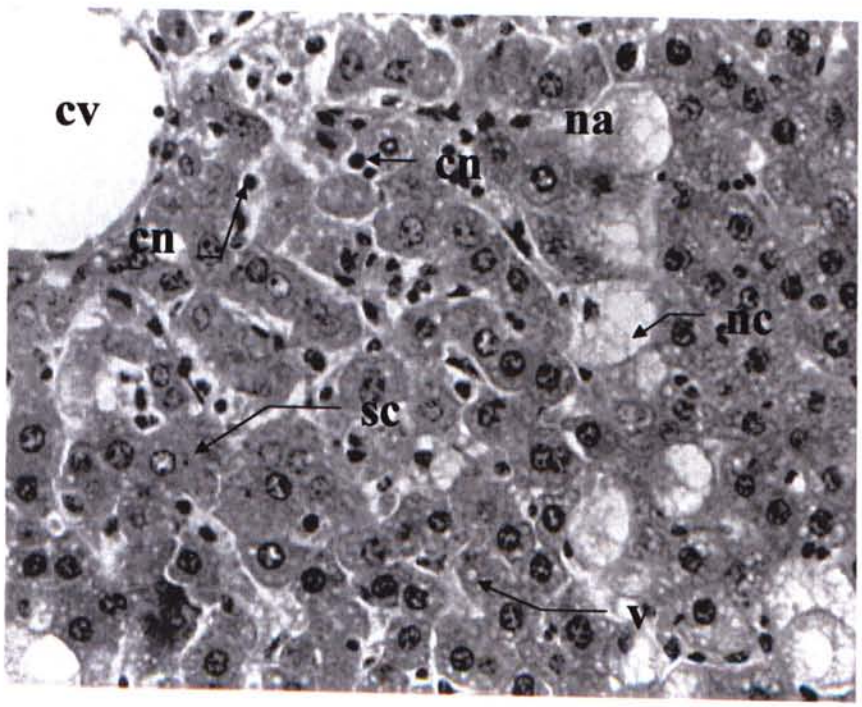
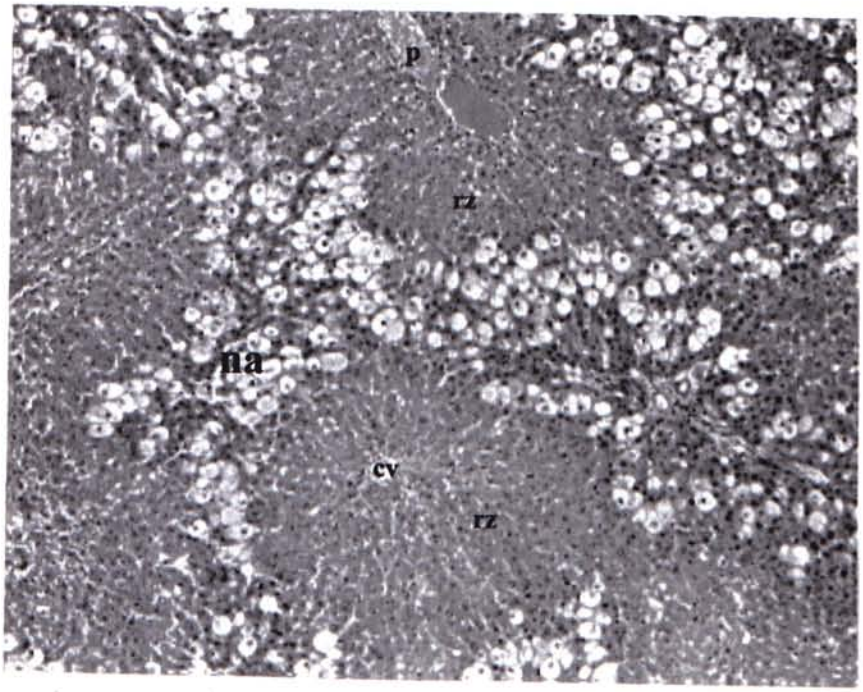

cv
na
cn
cn
nc
sc
v

Fig. 4.136 Effect of the NAC (30 mg/ml saline) on the liver of CCl_4-treated rat showing necrosis of hepatocytes with large range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; na, necrotic area; p, portal triad; rz, regeneration zone.
(60 ×, H & E)

Fig. 4.137 Effect of the NAC (60 mg/ml saline) on the liver of CCl_4-treated rat showing few necrosis of hepatocytes with very large range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; na, necrotic area; rz, regeneration zone.
(60 ×, H & E)


p
rz
na
cv
rz

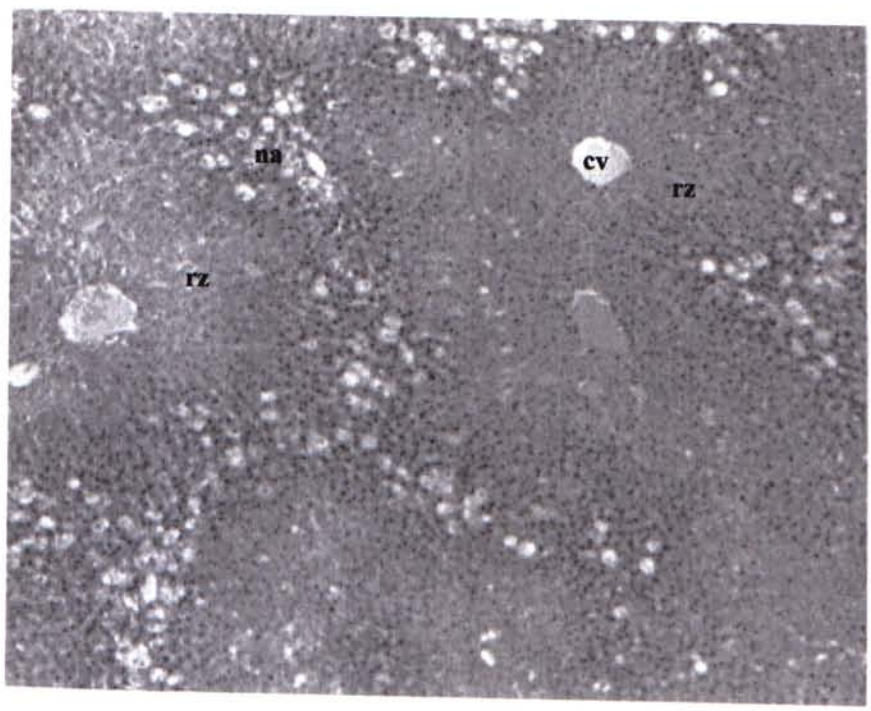
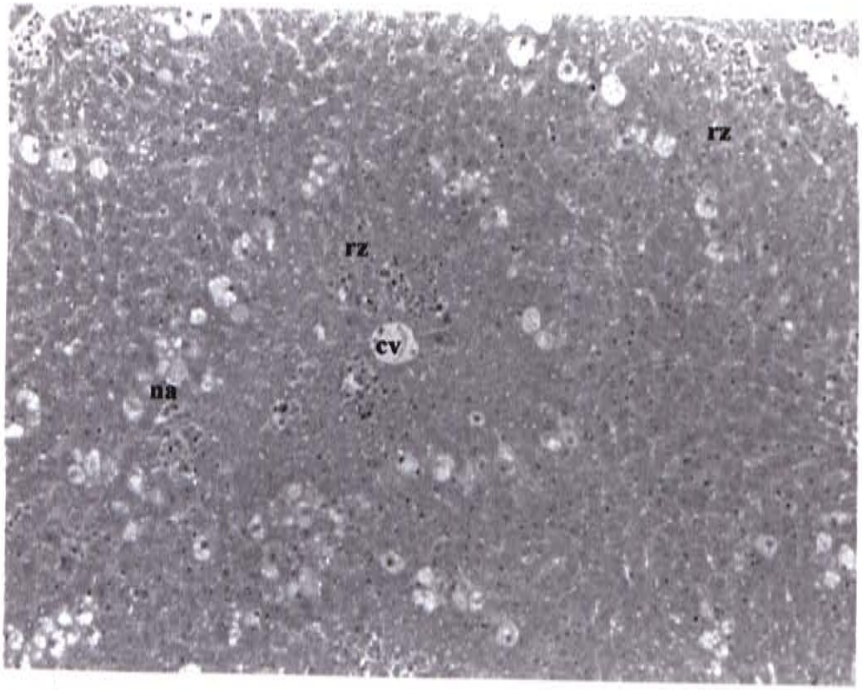

na
cv
rz
rz

Fig. 4.138 Effect of the NAC (60 mg/ml saline) on the liver of CCl_4-treated rat (duplicated) showing few necrosis of hepatocytes with very large range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; na, necrotic area; rz, regeneration zone.
(95 ×, H & E)

Fig. 4.139 Effect of the NAC (60 mg/ml saline) on the liver of CCl_4-treated rat (duplicated) showing massive necrosis of hepatocytes with very large range of regeneration zone around the central vein region as compared with the toxin control curative group. cv, central vein; cn, condensed nucleus; mf, mitotic figure; nc, necrotic cell; rz, regeneration zone; v, vacuolization.
(297 ×, H & E)


rz
rz
cv
na

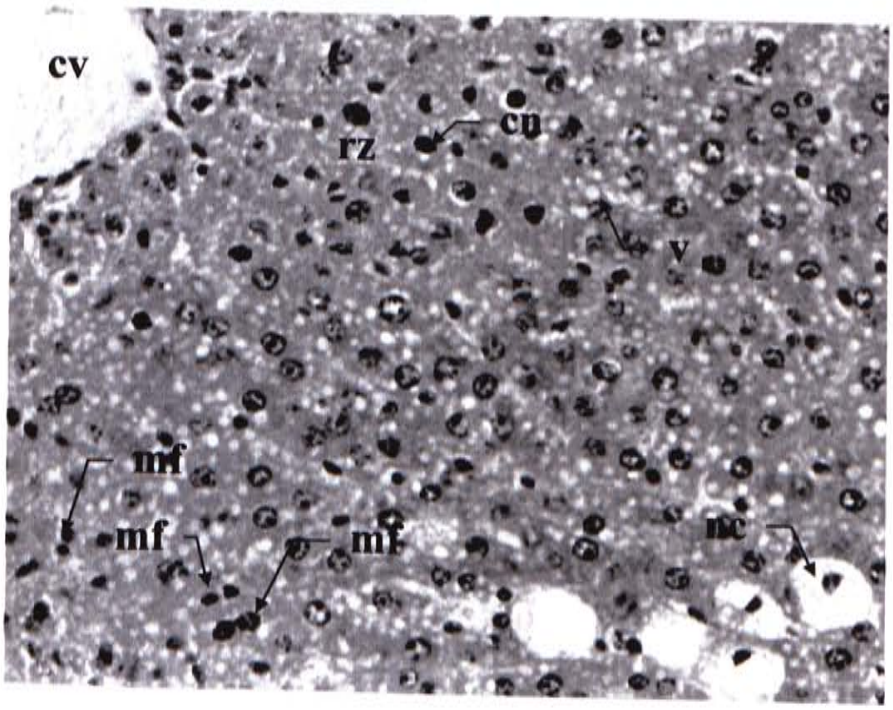
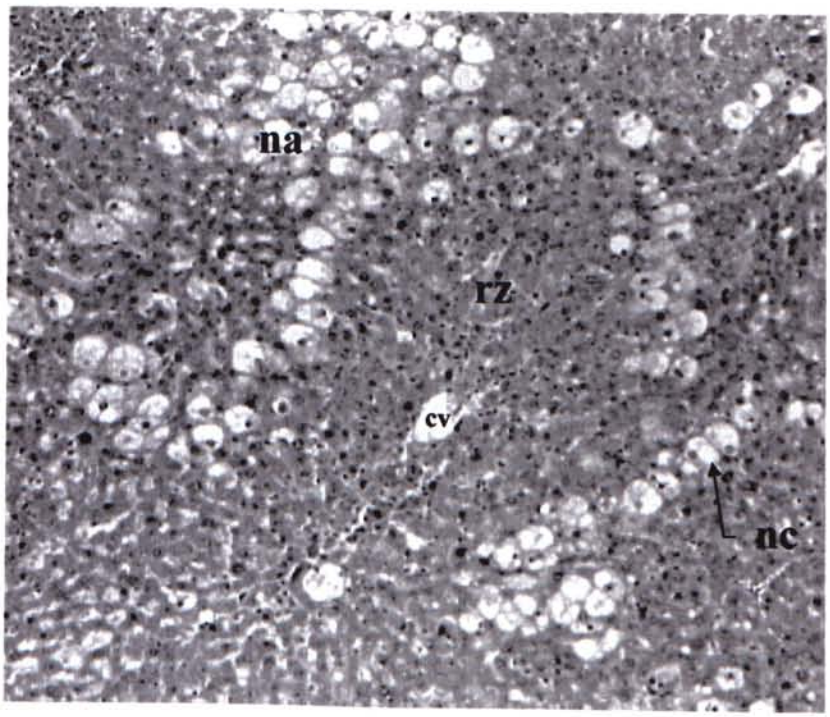

cv
rz
cu
v
mf
mf
mf
nc

Fig. 4.140 Effect of the seaweed's methanol extract (S#2: *S. henslowianum*, 30 mg/ml 25% DMSO) on the liver of CCl_4-treated rat showing necrosis of hepatocytes with large range of regeneration zone around the central vein region as compared with the toxin control curative group (DMSO 25% and saline treatment). cv, central vein; nc, necrotic cell; rz, regeneration zone.
(96 ×, H & E)

Fig. 4.141 Effect of the seaweed's methanol extract (S#2: *S. henslowianum*, 30 mg/ml 25% DMSO) on the liver of CCl_4-treated rat showing necrosis of hepatocytes with large range of regeneration zone around the central vein region as compared with the toxin control curative group (DMSO 25% and saline treatment). cv, central vein; mf, mitotic figure; nc, necrotic cell; rz, regeneration zone.
(194 ×, H & E)


na
rz
cv
nc

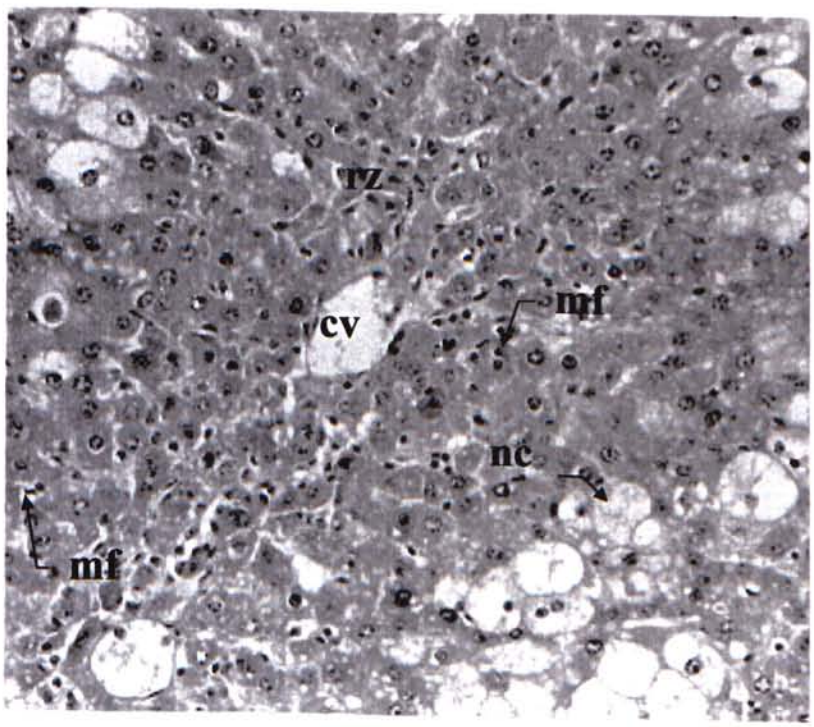
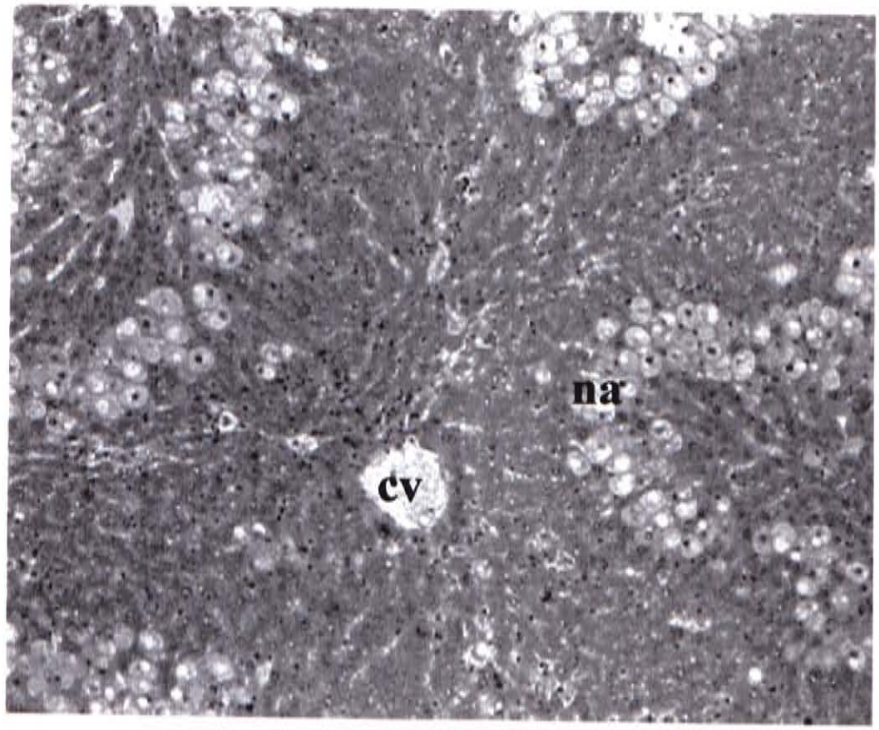

rz
cv
mf
nc
mf

Fig. 4.142 Effect of the seaweed's methanol extract (S#3: *Myagropsis myagroides*, 30 mg/ml 25% DMSO) on the liver of CCl_4-treated rat showing massive necrosis of hepatocytes around the central vein region as compared with the toxin control curative group (DMSO 25% and saline treatment). cv, central vein; na, necrotic area.
(97 ×, H & E)

Fig. 4.143 Effect of the seaweed's methanol extract (S#3: *Myagropsis myagroides*, 30 mg/ml 25% DMSO) on the liver of CCl_4-treated rate showing massive of necrosis of hepatocytes around the central vein region as compared with the toxin control curative group (DMSO 25% and saline treatment). cv, central vein; mf, mitotic figure; nc, necrotic cell; v, vacuolization.
(188 ×, H & E)


na
cv

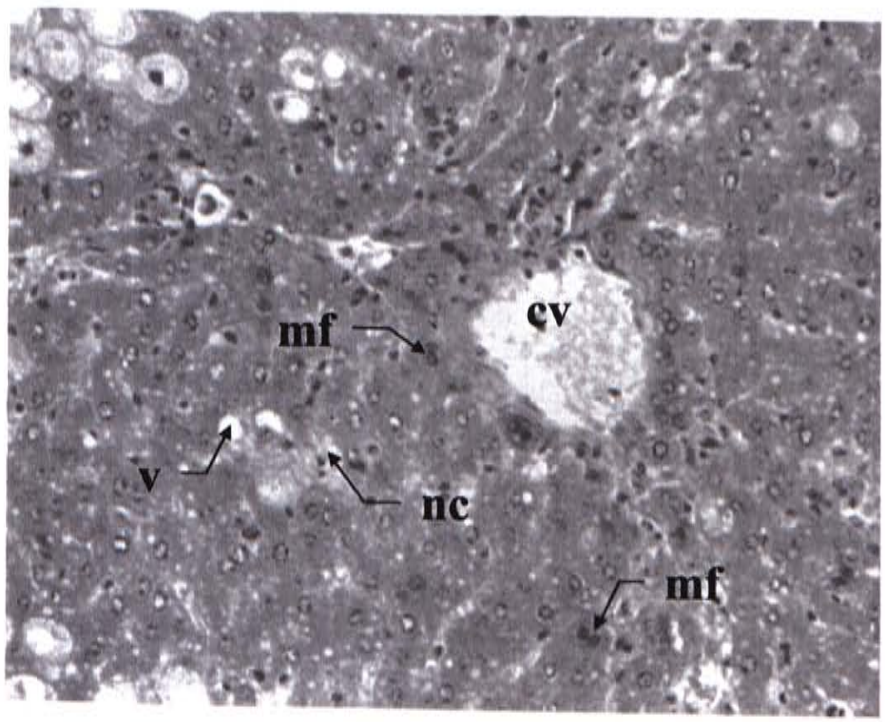
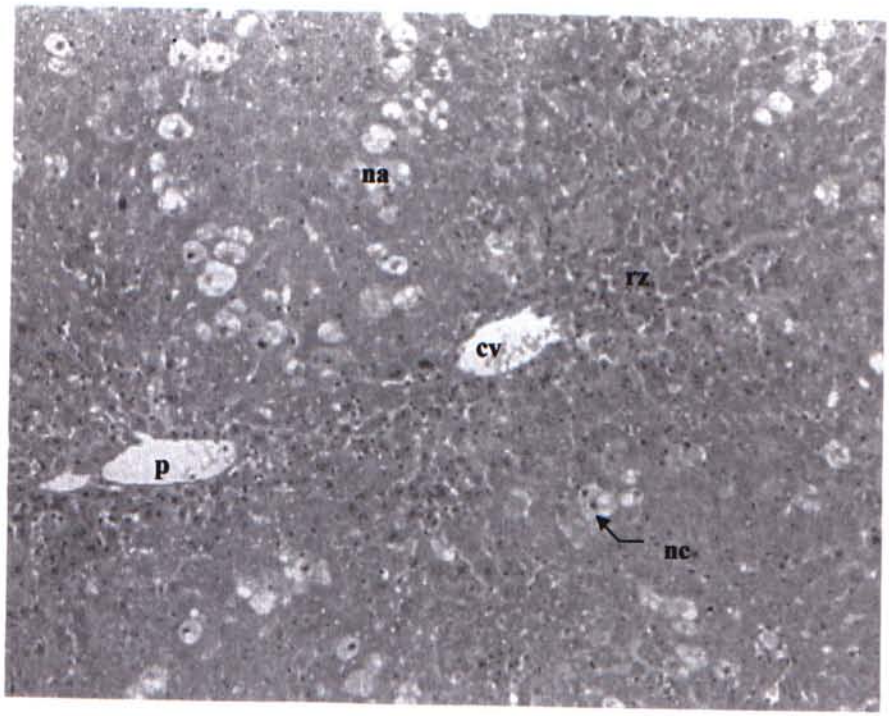
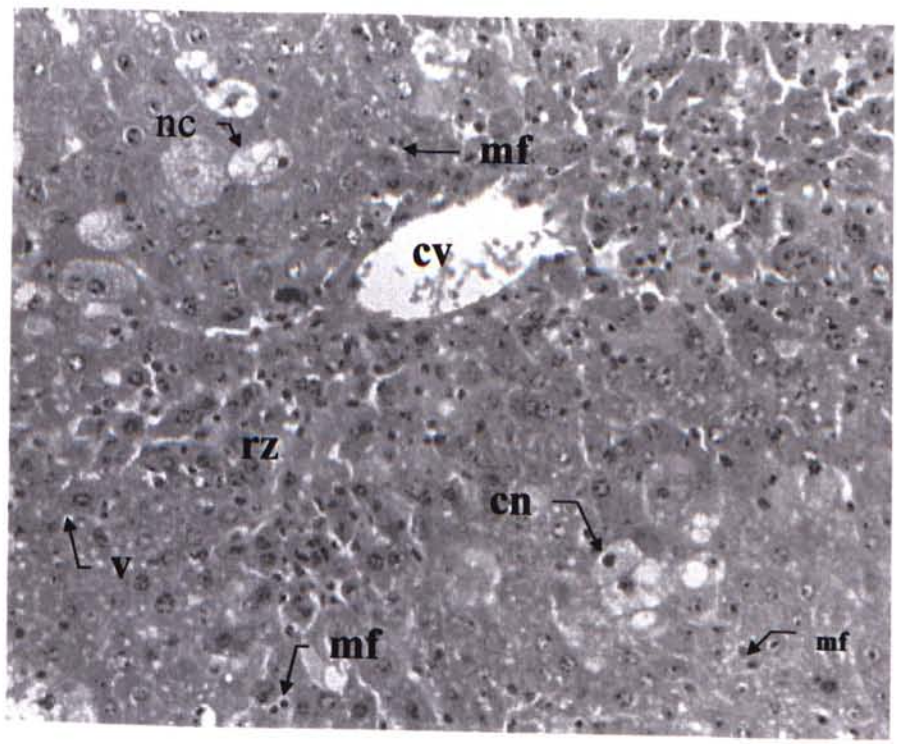

mf
cv
v
nc
mf

Fig. 4.144 Effect of the seaweed's methanol extract (S#4: *S. siliquastrum*, 30 mg/ml 25% DMSO) on the liver of CCl_4-treated rat showing few necrosis of hepatocytes with large range of regeneration zone around the central vein region as compared with the toxin control curative group (DMSO 25% and saline treatment). cv, central vein; na, necrotic area; nc, necrotic cell; p, portal triad; rz, regeneration zone.
(96 ×, H & E)

Fig. 4.145 Effect of the seaweed's methanol extract (S#4: *S. siliquastrum*, 30 mg/ml 25% DMSO) on the liver of CCl_4-treated rat showing few necrosis of hepatocytes with large range of regeneration zone around the central vein region as compared with the toxin control curative group (DMSO 25% and saline treatment). cv, central vein; cn, condensed nucleus; mf, mitotic figure; nc, necrotic cell; v, vacuolization.
(192 ×, H & E)


na
rz
cv
p
nc


nc
mf
cv
rz
cn
v
mf
mf